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Biological Control

ASSESSING HOST RANGES FOR PARASITOIDS AND PREDATORS USED FOR CLASSICAL BIOLOGICAL CONTROL: A GUIDE TO BEST PRACTICE

R. G. Van Driesche and R. Reardon, Editors

Forest Health Technology Enterprise Team—Morgantown, West Virginia

United States
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Agriculture

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Contents

Chapter 1. Introduction: Predicting Host Ranges of Parasitoids and Predacious Insects—What are the Issues? (by R. G. Van Driesche) 1

Chapter 2. The Effects of *Compsilura concinnata*, an Introduced Generalist Tachinid, on Non-target Species in North America: A Cautionary Tale (by J. S. Elkington and G. H. Boettner) 4

Chapter 3. Using the Scientific Literature to Estimate the Host Range of a Biological Control Agent (by D. P. A. Sands and R. G. Van Driesche) 15

Chapter 4. Analysis of Fauna in the Receiving Area for the Purpose of Identifying Native Species that Exotic Natural Enemies May Potentially Attack (by M. S. Hoddle) 24

Chapter 5. Behavioral and Physiological Processes Affecting Outcomes of Host Range Testing (by T. M. Withers and L. B. Browne) 40

Chapter 6. Parameters Used in Laboratory Host Range Tests (by R. G. Van Driesche and T. J. Murray) 56

Chapter 7. Overview of Testing Schemes and Designs Used to Estimate Host Ranges (by R. G. Van Driesche and T. J. Murray) 68

Chapter 8. Estimating the Host Range of a Thrips Parasitoid (by K. J. Froud and P. S. Stevens) .. 90

Chapter 9. *Microctonus* Parasitoids and New Zealand Weevils: Comparing Laboratory Estimates of Host Ranges to Realized Host Ranges (by B. I. P. Barratt) 103

Chapter 10. Evaluation of Lily Leaf Beetle Parasitoids for North American Introduction (by R. A. Casagrande and M. Kenis) 121

Chapter 11. Host Preference Testing for Parasitoids of a Eucalyptus Borer in California (by T. D. Paine, J. G. Millar, and L. M. Hanks) 138

Chapter 12. Estimating the Host Range of the Tachinid *Trichopoda giacomellii*, Introduced into Australia for Biological Control of the Green Vegetable Bug (by M. Coombs) 143

Chapter 13. Assessing Host Specificity and Field Release Potential of Fire Ant Decapitating Flies (by S. D. Porter and L. E. Gilbert) 152

Chapter 14. Determining the Host Range of *Aphantorhaphopsis samarensis*, a Specialized Tachinid Introduced Against the Gypsy Moth (by R. W. Fuester, K. S. Swan, M. Kenis, and F. Hérard) 177

Chapter 15. Predicting the Field Prey Range of an Introduced Predator, *Rodolia cardinalis* Mulsant, in the Galápagos (by C. E. Causton) 195

Chapter 16. Evaluating Host Range of *Laricobius nigrinus* for Introduction into the Eastern United States for Biological Control of Hemlock Woolly Adelgid (by G. Zilahi-Balogh) 224

Chapter 17. Conclusions (by R. G. Van Driesche) 240

CHAPTER 1. INTRODUCTION

PREDICTING HOST RANGES OF PARASITOIDS AND PREDACIOUS INSECTS—WHAT ARE THE ISSUES?

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GOALS FOR HOST RANGE TESTING

Estimating the likely nontarget impacts of agents released to suppress invasive plants has been legally required, to one degree or another, for many decades. Similar predictions were not formally required for introductions of parasitoids or predators of pest arthropods. That is now beginning to change. This book has as its goal an exploration of how such estimates can best be made. This requires overcoming a series of problems, some logistical, some technical, some tied to an unclear theoretical framework for the activity. In this book, the editors and authors have tried to address many of these needs, in some chapters as essays on important tasks that need to be achieved, in other chapters as case history explorations of how the tasks were done in particular cases. This book will not be the final answer, but we hope it might propel the search for such an answer along.

LEGAL REQUIREMENTS

Whether or not predicting the host ranges of parasitoids and predators is legally required varies among countries. There is an absolute requirement for such predictions in New Zealand and Australia, but not in most other countries. In the EU, there is a developing consensus that such information will be required, but in the United States legal authority is lacking to impose such a requirement. Rather, the degree of such an assessment currently depends on the agency of employment of the person importing the natural enemy, with more stringent requirements for federal employees.

Regardless of the current legal status quo in any particular country, there is a trend to impose such requirements. The role of this book is in part to shape how such requirements are written, by revealing some of the complexities in the process of making such estimates and highlighting the risks of making overly sweeping assumptions about the utility of laboratory test data.

PRACTICAL PROBLEMS

Some of the problems posed by estimating the host ranges of candidate entomophagous biocontrol agents relative to the fauna of the receiving country are purely practical, rather than theoretical. Compared to plants, the number of species in a native biota of insects can be overwhelmingly large, with hundreds, thousands, even tens of thousands of native species in the target pest's family in the receiving biogeographic region. Many of these are likely to have little or no information associated with museum specimens about such important matters as their biology, habitat, host plants, and so on. This double edged problem, too many species and too little information, can cripple efforts to rationally consider the impact of a new parasitoid or predator on such a group. Many species that would be desirable members of a host range test list may be impossible to find or, if found, information on how to rear them will be unavailable. Rearing difficulty is further compounded by the necessity of holding insects as reproducing colonies at great cost in labor, rather than as seeds or long-lived individuals as can be done for plants. Finally, the large numbers of species of many groups means that many other species exist that are unknown, especially if importations are being considered for tropical continental areas, and that modern molecular phylogenies of the relevant insect groups are less likely to be available than for plant groups.

ISSUES OF THEORY

Issues of theory also complicate the study of host ranges of entomophagous arthropods. For specialized herbivorous arthropods, host ranges seem to track plant taxonomy because that itself is often highly correlated to secondary plant chemistry, compounds often used by specialists for host plant recognition. No such simple framework exists shaping the host ranges of insect parasitoids – or at least work to date has not shown this to clearly be the case. Rather, parasitoids themselves are of two potentially different sorts – idiobionts and koinobionts – each of which may be tracking different things in selecting hosts. Idiobionts being outside of their hosts need not have the high level of physiological adaptation to manipulate living hosts' immune systems that koinobionts require. Rather, idiobionts may be freer to use a wider range of hosts and may be more shaped in their host choices by the habitats or host plants or type of plant structure (leafmine, gall, etc) in which they find hosts. Koinobionts, in contrast employ venoms, viruses and other devices to master their hosts' immune systems and as such may find it more feasible to learn to find taxonomically related host insects on novel plants than to dominate novel immune systems of less related hosts on familiar plants. Sorting this framework out is the big theoretical issue in predicting host ranges of entomophagous insects. Such information is needed to make sense of actual host range test designs and test lists.

TECHNICAL ISSUES

Many technical issues exist about which sorts of host range tests are most useful in predicting what entomophagous insects are likely to do after their release in a new region. How much weight should be placed on host finding versus host suitability? Should preference be considered a factor likely to protect nonpreferred species from attack or will the biological control agents find themselves accepting or rejecting hosts without other immediately available choices? Should data from small cage studies be viewed as a reliable indicators of host choice or should tests use large cages? If so, how large? Should biological control agents used in tests be naïve (no contacts with the target pest) or experienced? Hungry or satiated? Mated or unmated? Young or old? While much has been learned in the past 40 years about how parasitoids and predacious insects are influenced in their host foraging by such factors, synthesis of this information into an approach for host testing is just beginning.

PROCESS OF RESOLUTION

Present in this book are essay-style chapters that try to address a number of the above mentioned issues (Chapters 3-7). Other Chapters (8-16) present case histories in the belief that the particular stories they tell will cast light on methods, details, logical approaches that will have applications in other systems. Debate on many details of theory and practice will be needed to develop a consensus and a mature body of techniques for use in estimating host ranges of entomophagous arthropods. A new forum for such debate was begun in 2002 with the First International Symposium on Biological Control of Arthropods in Honolulu, Hawaii (USA). This series continues with the 2nd ISBCA scheduled to take place in Davos, Switzerland in September of 2005. Among the symposia to be held will be one debating issues affecting host range testing.

AGKNOWLEDGEMENTS

We thank the USDA Forest Service FHTET group in Morgantown, West Virginia, for funding the writing and printing of this volume, and to Mark Riffe, Intecs International, for formatting and layout. We look forward to the development of knowledge on host range testing and the eventual standard incorporation of such information into release decisions for new entomophagous insects.

CHAPTER 2. THE EFFECTS OF *COMPSILURA CONCINNATA*, AN INTRODUCED GENERALIST TACHINID, ON NON-TARGET SPECIES IN NORTH AMERICA: A CAUTIONARY TALE

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INTRODUCTION

Classical biological control has long been a principal weapon in the worldwide effort to combat the devastating effects of invasive species. Classical biological control involves locating natural enemies of invasive species in their native range and releasing them in the newly invaded habitat. The premise of classical biological control is that invasive species out-compete native species and become major pest problems in large part because they have become isolated from the suite of natural enemies that keep them in check in their native habitat. There have been many successes worldwide in the classical biological control of both invasive weeds and invasive arthropods. The advantages of classical biological control over any other approach are obvious and well known: the control exerted is typically permanent; it requires little or no further intervention; it is thus highly cost effective compared to mechanical removal or use of chemical pesticides, which must typically be applied repeatedly and are often infeasible in forests or other natural habitats.

Classical biological control of invasive weeds has had a long history of evaluating the host range of candidates for introduction. The obvious reason is that herbivorous natural enemies might become important pests of agricultural crops or other beneficial plants. In contrast, traditionally there has been little concern about the potential impacts on non-target native insects that might be caused by the introduced natural enemies of invasive arthropods. Indeed, the ability of natural enemies to attack native non-target species was viewed by many as a positive attribute (e.g., Culver, 1919; Webber and Schaffner, 1926). Native species might be pests in their own right, or, at the very least, they might provide a host reservoir that would maintain high densities of the natural enemy when densities of the target insect were low.

Until recently, there was little or no evidence that arthropod predators or parasitoids introduced as biological control agents against other invasive arthropods had had any important deleterious effects on non-target species. As a result, several authors have concluded that the technique is generally safe and unlikely to have significant effects on non-target organisms (Coulson *et al.*, 1991; Godfray, 1995). However, as pointed out by Howarth (1991), "absence of evidence is not evidence of absence" of such effects. In the last few years, several studies have elucidated negative impacts by a number of introduced agents (e.g. Obrycki *et al.*, 2000; Henneman and Marmot, 2001). Here we review our work on this topic focusing on the non-target effects of the generalist tachinid parasitoid *Compsilura concinnata* (Meigen), which was introduced to North America in 1906 primarily to control the gypsy moth, *Lymantria dispar* L. We have shown that this species is probably having a severe impact on a number of our native giant silk moths (Saturniidae) (Boettner *et al.*, 2000), which include our largest and most showy native Lepidoptera.

INTRODUCTION OF *C. CONCINNATA* TO NORTH AMERICA

The gypsy moth was introduced to North America in a suburb of Boston, Massachusetts in 1868. (Forbush and Fernald, 1896). By the late 1880s, it had become a serious defoliator in eastern Massachusetts, and this damage triggered a substantial eradication effort based on hand removal of gypsy moth egg masses and widespread application of lead and copper arsenate insecticides to infested trees. Despite this effort, the area infested by gypsy moth continued to expand across eastern New England in the first decade of the 20th century. The eradication effort was then abandoned, and in 1905, the United States Department of Agriculture embarked on a major effort to introduce predators and parasitoids of gypsy moth from it native range in Europe and Asia (Howard and Fiske, 1911). Eventually, ten species of parasitoids were successfully introduced and established on gypsy moth in North America (Elkinton and Liebhold, 1990). One of these species was *C. concinnata,* a tachinid with a very broad host range that has now been recovered from at least 180 species of Lepidoptera and Symphyta (Arnaud, 1978; Boettner *et al.*, 2000; Strazanac *et al.*, 2001). The broad host range of *C. concinnata* was well understood by the individuals involved in its dissemination, but its potential impact on non-target species was not a significant concern (Howard and Fiske, 1911; Culver, 1919; Webber and Schaffner, 1926). Introductions of *C. concinnata* and efforts to release it in other parts of the country against gypsy moth and other target species continued over much of the 20th century (Sanchez, 1996).

EFFECTS OF *C. CONCINNATA* ON GYPSY MOTH POPULATIONS

Despite the establishment of *C. concinnata* and nine other introduced parasitoid species, gypsy moth has continued to spread into southern and mid-western regions of the United States. Frequent outbreaks have continued to occur throughout the introduced range in northeastern North America. Even in its native range in Europe and Asia, where gypsy moth has a richer fauna of parasitoids and other natural enemies, outbreaks and defoliation by gypsy moth occur in many, but not all, regions with forests of appropriate host trees. Few long term studies have been done to document the impact of parasitoids on gypsy moth anywhere in the world, and their overall role in gypsy moth dynamics remains ambiguous (Elkinton and Liebhold, 1990). A study by Sisojevic (1975) in the former Yugoslavia appeared to show a classic host-parasitoid oscillation between gypsy moth and three tachinid species, of which *C. concinnata* was one. The level of parasitism recorded by Sisojevic and in other European studies was notably higher than that observed for the same parasitoid species on gypsy moth in North America (Elkinton and Liebhold, 1990). The most comprehensive study of parasitism in naturally occurring populations of gypsy moth in North America was conducted by Williams *et al.* (1992). In that study (Figure 1A), there was no hint of any direct or delayed density dependence of parasitism by *C. concinnata* on gypsy moth and overall levels of parasitism by this species never exceeded 20%. Parasitism by other species was also quite low and at best only weakly density dependent. The results of this study confirmed the conclusions drawn by earlier investigators: that parasitoids played a limited or equivocal role in the population dynamics of gypsy moth in North America (Campbell, 1975, Reardon, 1976; Elkinton and Liebhold, 1990).

Research involving experimentally created populations of gypsy moth in our laboratory (Gould *et al.*, 1990) produced quite different results from those of Williams *et al.* (1992). Our study involved collecting gypsy moth egg masses and placing them at densities that ranged from 40,000 to 1.4 million eggs per ha on hectare-sized plots in an oak-dominated forest in western Massachusetts, where the naturally occurring gypsy populations were very low and where there had not been any recent outbreaks of gypsy moth. Following egg hatch, we collected and reared gypsy moth larvae on a weekly basis and recorded parasitoid emergence. In contrast to the results reported by Williams *et al.* (1992), larval parasitism by *C. concinnata* was higher than that due to any other parasitoid or cause of death (including predation on larvae) and it was strongly density dependent (Figure 1B). We have confirmed these results in many subsequent experiments (e.g., Ferguson *et al.*, 1994). It is important to understand, however, that the data collected by Williams *et al.* (1992) (Figure 1A) represents variation in parasitism in

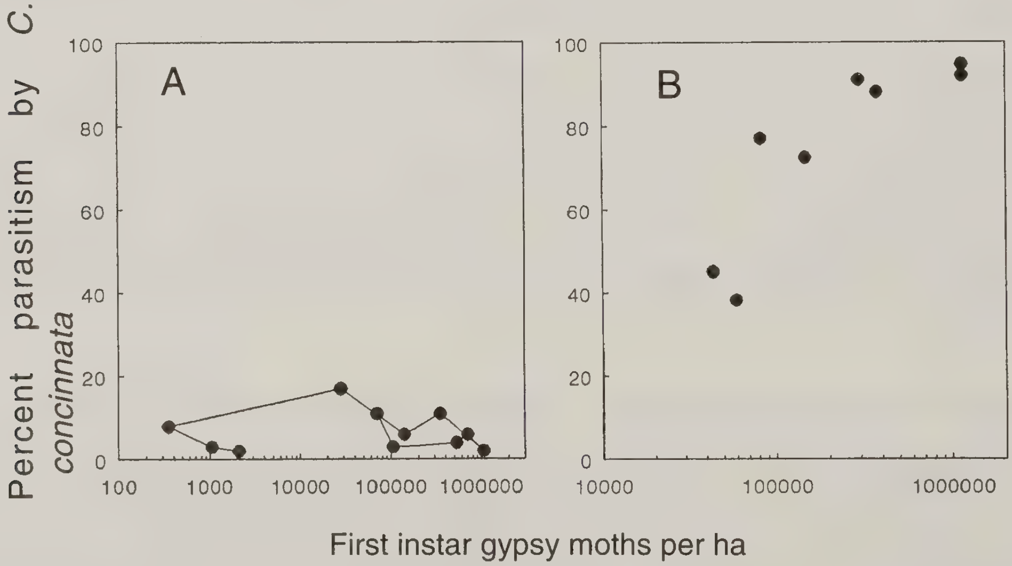

Figure 1. A) A time series of percent mortality caused by *C. concinnata* in a ten year study of gypsy moth in naturally occurring populations (Williams *et al.*, 1992). The solid line connects consecutive generations. Redrawn from Elkinton (2000). B) Percent mortality of gypsy moth caused by *C. concinnata in* a series of experimental populations created with different densities in the same year (Gould *et al.*, 1990).

a single population followed over several years (temporal density dependence), whereas the data from our experiments (Figure 1B) represents variation in parasitism among plots or populations all in the same year (spatial density dependence). Population ecologists have never been clear about the conditions under which spatial density dependence leads to temporal density dependence, but most agree that the latter is required for a natural enemy to stabilize the densities of its host. We do not know for sure why the levels of parasitism recorded in the two studies were so different, but we suspect that it is due to differences in spatial scale. In the hectare-sized, experimentally created populations, we suspect that *C. concinnata* aggregated to the higher density populations from forest areas outside the plots. In naturally occurring populations of gypsy moth, outbreaks and changes in density occur on a much larger spatial scale. The effects of aggregation from low density populations outside the outbreak area might be minimized or confined to the area near the perimeter of the outbreak. On the other hand, perhaps our study shows that *C. concinnata* plays a much more important role than we had realized in suppressing incipient outbreaks of gypsy moth on a small spatial scale.

EFFECTS OF *C. CONCINNATA* ON GIANT SILK MOTHS

The large impact by *C. concinnata* on gypsy moth that we recorded in the experiments reported by Gould *et al.* (1990) made us wonder what impact this species might be having on other native Lepidoptera, particularly those such as giant silk moths (Saturniidae) that finish larval development in late summer. *Compsilura concinnata* is a multivoltine insect (Culver, 1919); it completes a first generation on gypsy moth and two or three subsequent generations on other Lepidoptera whose larvae are present in late summer. This fact may link the dynamics of these different species. The numbers of *C. concinnata* available to attack gypsy moths in the spring will be determined by the abundance of late summer hosts for *C. concinnata*. Similarly, the attack rates by *C. concinnata* on late summer Lepidoptera may be determined by the abundance of gypsy moths.

Cecropia moths, (*Hyalophora cecropia* L.) like other giant silk moths, became notably rarer in the northeastern United States in the late 20th century than they used to be (Schweitzer, 1988; Tuskes *et al.*, 1996). There exist no data to prove this fact, but anecdotal descriptions by collectors of local densities in the 19th century far exceed densities that exist today (Elliot and Soule, 1902; Smith 1908). Several hypotheses have been advanced to account for this decline (Schweitzer, 1988; Tuskes *et al.*, 1996). These ideas include the widespread use of DDT to suppress gypsy moth in the 1960s, the decline of host trees due to urban development, and the deleterious effects on moth mating of mercury vapor street lights. There was no concrete evidence for or against any of these hypotheses, but we considered them unlikely (Boettner *et al.*, 2000). Applications of DDT to forests in the northeast ceased in the 1960s and even at their height never encompassed more than a small fraction of the total forest area. Application of replacement pesticides, such as carbaryl, to forest tracts ended in New England the early 1980s. No resurgence of giant silk moths has been evident. Research on the effects on native Lepidoptera of pesticides applied to forests for gypsy moth control suggests that the impacts are quite ephemeral, rarely lasting beyond the year of application (Sample *et al.*, 1993). As for host availability, different silkmoth species feed on different, but common, deciduous tree species. Despite urban/suburban development, total forest cover in New England has increased over the past century due to abandonment of agriculture in the region (Foster, 1995). Thus total host availability for silkmoths on a regional basis should have increased, not deceased. Mercury vapor lamps have been used nationwide, yet the decline in silk moths was noted only in the northeast (Tuskes *et al.*, 1996)

Our experience documenting the large impact of *C. concinnata* on experimentally created populations of gypsy moth (Gould *et al.*, 1990) lead us to propose an alternative hypothesis: that populations of giant silk moths have been suppressed by *C. concinnata.* Giant silkmoths have larval stages that last as long as 60 days, and the larvae are present in late summer when those of other Lepidoptera are scarce. We hypothesized that *C. concinnata* densities would be especially high in the northeast, where first generation numbers would build up on highly abundant gypsy moths and then move to attack other species in late summer.

To test this hypothesis, we deployed 500 first instar cecropia moths on trees at the same site in western Massachusetts as our earlier work on *C. concinnata* impact on gypsy moths (Gould *et al.*, 1990). We put them out on understory black cherry trees (*Prunus serotina* L) at a density of five per tree on 100 trees spaced at 5-20 m intervals along four transects across the 64 ha forest. We followed these larvae continually through the entire larval stage in order to get a measure of total larval survival. The initial density per tree of the larvae that we deployed was comparable to the density (two to six eggs) of naturally occurring cecropia moths (Tuskes *et al.*, 1996). In addition, we reared other larvae in the laboratory and placed cohorts of 100 larvae on nearby black cherry trees at five larvae per tree using the same instar as the larvae that we were monitoring in the field for overall survival. Approximately one week later, we retrieved this second group of larvae and replaced them with another laboratory-reared cohort. The retrieved larvae were returned to the laboratory and reared in cups on foliage and monitored frequently for mortality and parasitoid emergence. In this way, we were able to record attack rates by parasitoids instar by instar as the larval stage progressed.

None of the original 500 cecropia larvae that we deployed in the field survived to the pupal stage (Figure 2). In fact, none survived longer than 40 of the approximate 60 days required to complete larval development. The vast majority of this mortality was caused by *C. concinnata.* It caused a cumulative mortality of 81% among the first three instars and was by far the largest cause of death (Table 1). The total mortality caused by *C. concinnata* that we documented would have been even higher had we been able to record attacks on later instars. Unfortunately, our laboratory-reared colony of cecropia larvae became infected with a pathogen so we did not have fourth and fifth instars to deploy. We know from other subsequent research (Kellog *et al.*, 2003) that levels of parasitism by *C. concinnata* on fourth and fifth instar cecropia are even higher than on the first three instars. Thus we conclude that the levels of parasitism by *C. concinnata* in our field populations were at least 81% and were probably a good deal higher.

We wished to compare our results to previous studies of cecropia moth larval survival and found that no such studies existed. The only data on cecropia moth survival and causes of death that we found was of cecropia pupae in Illinois (Marsh, 1937). Using this information along with data on cecropia fecundity and arbitrarily assuming 100% survival of egg and adult stages, we calculated the larval survival needed to maintain cecropia populations at a constant density (dotted straight line in Figure 2). Our observed survival was much less than that. If we had been able to incorporate the actual mortality rates of egg and adult stages, the required level

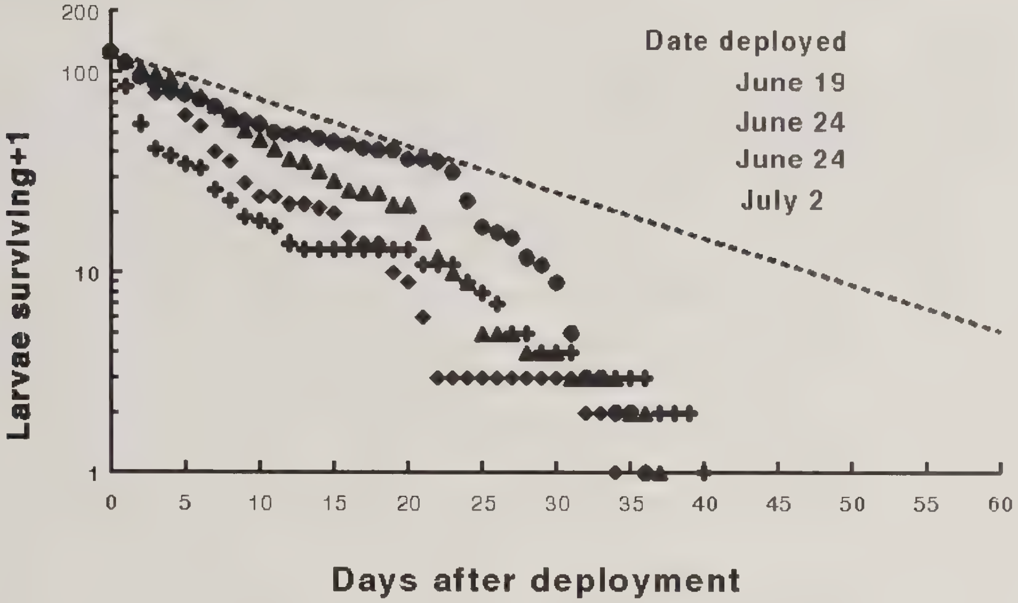

Figure 2. Survivorship curves ($\log_{10}$ numbers plotted vs. time) for deployed *H. cecropia* recorded from daily observations. Dotted line represents an estimate of the required survival for a population to experience no change in density based on data collected by Marsh (1937). Reprinted with permission from Boettner *et al.* (2000).

Table 1. Causes of death for larval cecropia moth and the percentage observed dying at each stage among cohorts of larvae released and recovered from the field. [a] Reprinted with permission from (Boettner *et al.*, 2000).

	Observed Field Mortality				**Rearing Mortality**		
Stage	**No. of larvae deployed**	**Spiders (%)**	**Stink bugs: Pentatomid (%)**	**Days in field**	**No. of larvae recovered for rearing**	**Ichneumonid *H. fungitivus* (%)**	**Tachinid *C. concinnata* (%)**
1st instar	100	5	4	7	54	1.9	13
2nd instar	100	4	0	5	40[b]	0	27.5
3rd instar	100	4	0	6	40	0	70
Totals[c]	300	12.4	4		134	1.9	81.1

[a] Compiled from daily checks and additionally rearing out the survivors of each instar.

[b] One second instar escaped during rearing and is not included in this total.

[c] Total percentage mortality calculated as $1-(1-m_1)(1-m_2)(1-m_3)$ where $m_i =$ the fraction dying during instar i.

of survival of larval cecropia for population stability would have to be even higher. Our conclusion is that no cecropia population can long persist if it sustains the level of mortality that we observed in our field experiment. We recognize, of course, that the rates of parasitoid attack are likely to diminish from the levels that we observed as the density of cecropia declines; indeed, that is the only way that cecropia can persist in nature. We point out, however, that declines in attack rates with host density by generalist natural enemies such as *C. concinnata* are not inevitable because the density of *C. concinnata* is unlikely to be linked to that of particular host species, especially those such as cecropia moth, which are relatively sparse compared to other lepidopteran hosts.

Nevertheless, we have wondered whether aggregation responses by *C. concinnata* may have elevated the levels of parasitism we observed on our experimentally created cecropia populations in a manner similar to what occurred for our experiments with gypsy moth (Gould *et al.*, 1990). The density of five larvae per tree on trees spaced at 5-20 m intervals may be comparable to what occurs in nature for cecropia moth eggs (Tuskes *et al.*, 1996) but is higher than that observed for late instars, at least now that natural densities have declined. To test this idea, we have run follow-up experiments on Cape Cod with the polyphemus moth, *Antheraea polyphemus* (Cramer*)*. We compared *C. concinnata* attack rates on larvae deployed at densities of one per tree vs. five per tree, with trees spaced at least 20 m apart on 12 km transects (Boettner and Elkinton, unpublished). As we hypothesized, attack rates of larvae deployed one per tree were lower than on larvae deployed five per tree, but they were still very high. Overall attack rates by *C. concinnata* on polyphemus larvae in this experiment were even higher that what we had recorded for cecropia moth (Boettner *et al.*, 2000) and on some cohorts reached 100% after only 6-7 days in the field!

EFFECTS OF *C. CONCINNATA* ON OTHER GIANT SILK MOTHS

In a follow-up study to ours, Kellogg *et al.* (2003) deployed luna moth larvae (*Actias luna* L) in Virginia. Like us, they found that *C. concinnata* dominated the parasitoid fauna emerging from these larvae (accounting for 78% of the parasitoids reared), although the rates of attack were not quite as high as we reported on cecropia in Massachusetts. They also found that up to 47% of the *C. concinnata* that emerged from these larvae were hyperparasitized by trigonalid wasps, a species we have not seen in Massachusetts. Both we and Kellogg *et al.* (2003) also released and recovered single cohorts of promethea moth (*Callosamia promethea* [Drury]). We found 67% parasitism by *C. concinnata* among 117 larvae that were deployed in the field and recovered after 6 or 8 days (Boettner *et al.*, 2000). In contrast, Kellog *et al.* (2003) found no parasitism by *C. concinnata*, but theirs was a very small sample (18 larvae recovered), so any comparisons with our findings with this species are quite tentative. Finally, we collected 50 naturally occurring pine barrens buck moth larvae (*Hemileuca maia maia* Drury) on Cape Cod, Massachusetts, a species listed as threatened (Boettner *et al.*, 2000). We found that *C. concinnata* had parasitized 36% of them. This finding was very similar to the 30% parasitism reported by Stamp and Bowers (1990) in the closely related *Hemileuca lucina* Edwards, which they collected in central Massachusetts. In all of these examples, *C. concinnata* was causing higher levels of parasitism than any other parasitoid species. Thus, while more intensive studies would be required to elucidate the dynamics of any of these species, it is clear that *C. concinnata* has become a major, if not dominant source of mortality that was superimposed on whatever mortality factors were already governing the dynamics of these species before the introduction of *C. concinnata*. For this reason, we believe *C. concinnata* is the most likely cause of the reported decline of giant silkmoths in the northeastern United States.

CONCLUSIONS

The results of our studies with *C. concinnata* are sobering to anyone concerned with conservation of our native insect fauna. Other studies with other introduced biological control agents are starting to tell a similar story (e.g., Obrycki *et al.*, 2000; Henneman and Marmot, 2001). Perhaps the most striking fact to us is how little we know about the population dynamics and impact of natural enemies for any of our native species. Ours was the first study of its kind on cecropia moth, one of our largest and most showy native Lepidoptera. We know even less about the changes in density and their causes for basically all other native insect species. Even for species such as gypsy moth that have been studied intensively by generations of researchers, our understanding of the impact of particular natural enemies, such as *C. concinnata,* remains very imperfect. The reason for this state of affairs is that research on insect population dynamics is very expensive and difficult. It requires a long-term commitment by investigators and has never been a priority for the state or federal agencies charged with managing our forests and natural habitats.

We believe that the community of scientists involved in biological control introductions must take the lead in establishing guidelines and standards for host range testing to make sure that generalists such as *C. concinnata* are no longer introduced (Simberloff and Stiling, 1996; Strong and Pemberton, 2000; Louda *et al.*, 2003). According to Nechols *et al.* (1992), generalist predators and parasitoids with a wide host range should no longer pass established protocols for United States introductions. However, these protocols are voluntary for biological control agents released to control invertebrates. Despite tighter standards and wider concern about this issue, release of generalist parasitoids with broad host ranges has continued. For example, personnel involved in gypsy moth control (Anonymous, 2000) have continued to release *Pimpla disparis* (Viereck), an inchneumonid pupal parasitoid with as broad a host range as *C. concinnata* (Schaefer *et al.*, 1989). We also believe, however, that we must strike a balance between preventing such introductions and restrictions on introductions or requirements for host-range testing that are so restrictive and expensive that classical biological control becomes infeasible (Van Driesche and Hoddle, 1997). The overall approach of biological control remains the most important weapon we have against many invasive species. We must develop protocols and guidelines that allow us to use this tool more wisely than we have in the past.

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CHAPTER 3. USING THE SCIENTIFIC LITERATURE TO ESTIMATE THE HOST RANGE OF A BIOLOGICAL CONTROL AGENT

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INTRODUCTION

Early in any classical biological control project, researchers are likely to compile lists from the literature of the parasitoids and predators recorded as attacking the target pest in its native range or in other locations where it has invaded. One of the first tasks in such a project is to review this information and identify any species that seem promising enough for study as potential biological controls. One aspect of that synthesis of the literature is to form a preliminary assessment of the likely host range of any listed parasitoid or predator. In this chapter, we suggest how this process can be done effectively and point out some of the pitfalls likely to be encountered.

SOURCES OF INFORMATION

Journal articles, books, internet sources, and specimens in museum collections are the major sources of information on the likely host range of a new biological control agent. However, such information will be somewhat biased as parasitism (or predation) is more likely to have been recorded on pest species than on non-pest species. Information from computerized databases alone is not sufficient to identify all recorded hosts. Literature prior to 1971 is not covered in such databases and may contain useful information about hosts or prey of parasitoids and predators. Alternate hosts of natural enemies, for example, were often studied because such species could be used to rear parasitoids in the laboratory (DeBach and Schlinger, 1964).

In other cases, non-pest species attacked by natural enemies were studied as potential supplementary hosts for field populations of the natural enemy of interest (Huffaker and Messenger, 1976).

Major sources of information (scientific journals, conference proceedings, and reports) from 1971 forward are available on computer and are abstracted in such tools as CAB and AGRICOLA, available in most major university libraries. Older literature (back to 1913) can be assessed by using printed copies of the abstract journal *The Review of Applied Entomology*. Information since 1981 is found in *Biological Control News and Information* (published by CAB). Books of value include historical compilations of biological control programs in various regions (e.g., Clausen, 1978; Cameron *et al.*, 1989; Waterhouse, 1998; Waterhouse and Sands, 2001; Mason and Huber, 2002, among others), host/parasitoid catalogues such as Thompson and Simmonds (1964-1965), and regional catalogues of the major insect orders that contain natural enemies, such as Krombein *et al.* (1979)'s review of Hymenoptera in North America, Gibson *et al.* (1997)'s treatment of genera of Nearctic Chalcidoidea, and Arnaud (1978)'s catalog of the North American Tachinidae.

At the start of any effort to compile the literature on a candidate natural enemy, its current taxonomic status and any past synonyms must be determined. Sometimes, an agent that has been already introduced against the same or different pest was known by a different scientific name. Unrecognized synonyms can cause important information to be overlooked.

Taxonomists specializing on local groups of agents may often be based in museums in the countries where the target and potential agents are native species. Museum curators are often aware of little-known publications, such as natural history society newsletters or museum reports that may contain useful information on agent-host associations. Such printed sources are often missed, especially if they were published in unfamiliar languages. The curators may also be able to provide access to host or prey data on labels attached to specimens held in other official or private collections.

Many museums have computerized the information on the labels of the specimens in their collections, including information about hosts or prey that is rarely published. Such records may be helpful in identifying hosts for a particular parasitoid because voucher specimens of field-collected parasitoids, sometimes with the hosts from which they were reared, are often submitted to taxonomists to confirm identities and be retained in the museum's collection. Finally, email group lists may be useful tools for collecting scattered information about host records for species of interest.

BIOTYPES AND HOST RANGES

Another general problem with literature and museum records is that unrecognized biotypes may exist. Either the host or parasitoid might actually consist of several distinct populations that vary biologically but are lumped under a single taxonomic name. If for example, a host consists of a series of such populations, a parasitoid may only attack some of them. Conversely, a parasitoid may exist as several distinct biotypes, each of which attacks somewhat different hosts.

Unless biotypes are distinguished, a complex of narrowly specific parasitoids will incorrectly be viewed as one widespread, more polyphagous species. For example, molecular analyses have now shown that the braconid *Microctonus aethiopoides* Loan, used for control of various forage weevils, consists of at least two biotypes, one associated with weevils in the genus *Sitona* and the other with weevils in the genus *Hypera* (Vink *et al.*, 2003). Similarly, there are two biotypes of *Comperiella bifasciata* Howard, each adapted to parasitize only one of two closely related scales. The yellow scale biotype of *C. bifasciata* successfully parasitizes yellow scale, *Aonidiella citrina* (Coquillet), but it does not develop on red scale, *Aonidiella aurantii* (Maskell). The yellow scale biotype will oviposit in red scales, but many of the parasitoid eggs and some larvae become encapsulated, and no parasitoids develop (Brewer, 1971). By contrast, the red scale biotype of *C. bifasciata* successfully parasitizes up to 80% of adult females of *A. aurantii* (Smith *et al.*, 1997).

Because of the potential for biotypes, host range testing should be done with the exact geographic population of parasitoid that will be released; otherwise, testing and releases may employ different biotypes, which might differ in their host ranges.

ERRORS AND OUTLIERS

Misidentifications of parasitoids or of their associated hosts sometimes appear in the literature or on the labels attached to specimens in museums. For material in collections, the identities of the parasitoid and host can be checked with appropriate taxonomists if physical specimens are available. Whenever possible, this should be done before the host records are accepted as valid. The validity of host records in the literature is harder to assess as specimens either may not be available at all, or considerable effort and international contacts may be required to locate specimens and have them shipped to a competent taxonomist for identification.

In the process of assembling host records for a parasitoid or predator of interest, the use of some species as hosts will be supported by many records. This redundancy of information increases confidence in the validity of the information. Brief or unique records that are the only mention of a given species as a host for the parasitoid need confirmation. A single rearing record is, naturally, less certain than records for hosts from which the parasitoid has been reared repeatedly, over many dates and locations. Similarly, if two or more authors independently report attack by the parasitoid on a host, one has greater confidence in the record than if only a single report is found in the literature.

Some host records may be found that seem anomalously distant from the rest of the known hosts of a parasitoid. In some cases, outlier host records may simply be misidentifications, as discussed above. For example, the report of *Cotesia glomerata* (L.) from the arctiid *Pericallia ricini* F. (Ghosh, 1998) is almost certainly an error because the published picture of the cocoon (with a halo) and the solitary nature of the parasitoid brood do not match *C. glomerata*, which is gregarious and has cocoons that do not have a halo.

Less clear would be the case of *C. glomerata* specimens reportedly reared from *Colias lesbia* (Fab.) in Argentina by Sharkey *et al.* (2000). This record is an outlier in two dimensions: first, this is the only record of this species attacking hosts in the subfamily Coliadinae (within the Pieridae); second, the parasitoid cited is not native to South America, and while it has been released in Chile, it has not released in Argentina and is presumed to still be only found west of the Andes. Is this record accurate? The *C. glomerata* species group includes a number of hard-to-distinguish species. At a minimum, this record needs a second opinion from another braconid specialist and perhaps even the use of some recently developed molecular markers. The later would require the recollection of fresh material, preservation in 100% alcohol, and shipment to the laboratory with the necessary DNA markers. Similarly, there is a record of *C. glomerata* from *Ascia monuste* (L.) (a pierine) in Brazil (Scaglia *et al.*, 2003). This is an extreme outlier from the geographic point of view, for the reasons just mentioned. Again, such a record needs confirmation before it can be accepted as valid.

Other seemingly anomalous host records may represent "parasitoid errors" that can provide interesting insights into the factors affecting host selection by the parasitoid. *Cotesia glomerata*, for example, normally parasitizes pierine butterfly larvae associated with mustard plants. Attack of this species on *Aporia crataegi* L. (Jiang ShuangLin, 2001) is unusual in that the host of this pierid butterfly is a rosaceous plant (*Crataegus azarolus* L.) rather than a mustard oil crucifer; yet, this record is well substantiated. The chemical composition of volatiles from this host/plant complex relative to that of the typical host (*Pieris*/cabbage) would be of interest to see if some unsuspected similarity might exist. Even more unusual is the attack of *C. glomerata* on the tenthredinid sawfly *Athalia proxima* Klug (Johri *et al.*, 1992), a species quite distant from the parasitoid's normal pierine lepidopteran hosts. This record, if accurate, may be a "parasitoid mistake" caused by the fact that this sawfly feeds on mustard oil plants, and thus its plant-herbivore chemical signature may resemble that of this parasitoid's usual hosts.

USING NEGATIVE DATA TO YOUR ADVANTAGE

Sometimes the absence of parasitism under field conditions can be as useful as positive records from the literature. Information may exist that suggests that either a parasitoid species does not forage in a given habitat, or that certain hosts are in extensive contact with the parasitoid but not attacked. Recognizing and using such negative data can help in better estimating which, if any, of the native insects in an area where a parasitoid is proposed for release might be at risk.

HABITATS NOT USED BY THE PARASITOID

Parasitoids will only put native species at risk if they search the habitats of those species. Consequently, understanding the habitat limits of a parasitoid is helpful in predicting its host range. Literature reports or work done during the foreign collecting phase of a project may provide information on the physical characteristics of a parasitoid's habitat (see the chapter by Casagrande and Kenis in this volume). Knowing which habitats a parasitoid enters can be used to identify non-target organisms that might come in contact with the agent. For example, because the braconid *C. glomerata* forages in sunny meadow habitats but not shady forest ones, *Pieris napi* *oleracea* Harris is attacked in the northeastern United States by this introduced parasitoid, but *Pieris virginiensis* Edwards, an obligate forest butterfly, is not (Benson *et al.*, 2003ab).

In addition to a parasitoid or predator's general environmental adaptation (in terms of elevation, rainfall, temperature extremes, sun versus shade, etc), entomophagous species can be adapted to occupy certain plant species or plant communities, which may be preferred or even essential for the natural enemy's survival. For example, some species of phytoseiid mites are found only on a single species of tree in Australian rainforests (Beard and Walter, 2001). As such, prey species on other kinds of trees or in non-forested habitats would not come in contact with such predators were they used in a biological control program. Similarly, some species of whitefly parasitoids in the genera *Eretmocerus* and *Encarsia* show a marked preference to search some species of crop plants and not others for hosts (Goolsby *et al.*, 1998).

Field observations in a parasitoid's native range can be used to determine a species' habitat preferences. Kuhlmann *et al.* (2000) surveyed the habitat preferences of mirid plant bugs and their parasitoids in a variety of habitats in Europe as part of the process of selecting *Lygus* bug parasitoids suitable for introduction into North America against agricultural pest *Lygus* species. Sands and Coombs (1999) noted adaptation of the tachinid *Trichopoda giacomellii* (Blanchard) to grasslands and crop habitats in Argentina when evaluating the suitability of the parasitoid as an agent for controlling *Nezara viridula* (L.) in Australia. One non-target species tested, *Alciphron glaucas* (Fabricius), a species related to *N. viridula*, was not considered to be at risk from attack by *T. giacomellii*, even though it supported complete development by the parasitoid in the laboratory, because (unlike *N. viridula*) the nontarget species was adapted to rainforest habitats.

HOSTS IN CONTACT WITH THE PARASITOID BUT NOT ATTACKED

Herbivores in contact with a parasitoid but not attacked can tentatively be listed as not in the parasitoid's host range. This assumes that these herbivores' ranges overlap geographically with that of the parasitoid and that adequate surveys to detect parasitism of the relevant species have been done. Using such negative data, one can narrow the scope of species requiring formal testing in laboratory host range tests. Such observations can be helpful in recognizing parasitism that sometimes results from 'laboratory artefacts' (sensu Sands, 1993), in which confinement in cages in laboratory host range tests can cause nontarget species to be parasitized. For example, the lack of attack by the thrips parasitoid *Thripobius semiluteus* Boucek on the nontarget thrips *Hercinothrips bicinctus* (Bagnall) in the field in Australia allowed Froud and Stevens (this volume) to conclude that the low level of parasitism seen on *H. bicinctus* in their laboratory host range trials was really an artefact of confinement.

ARE HOST RANGES OF NEAR RELATIVES PREDICTIVE?

Frequently, in the course of foreign exploration for a classical biological control project, previously unknown parasitoids are found. Some insight into the possible hosts of such an undescribed species of parasitoid can be gained from information about the hosts of other parasitoids in the same genus. Species of *Cotesia* (Hymenoptera: Braconidae) appear to be predominantly parasitoids of lepidopteran larvae. Similarly, all *Aphidius* species will be aphid parasitoids. However, the breadth of a new species host range is not readily predicted from its congeners, since many genera contain both host-specific and generalist species. Consequently, a candidate with an unknown host range should not be excluded from further consideration and testing simply because some of its congeners have broad host ranges (Sands, 2000). For example, the South American tachinid *T. giacomellii* is a narrowly host-specific parasitoid of the green vegetable bug, *N. viridula,* that develops only on a few related pentatomids (Liljesthrom, 1980), but other species of *Trichopoda,* for example *Trichopoda pennipes* (Fabricius), have wider host ranges (Huffaker and Messenger, 1976), attacking species in several families of Homoptera (Pentatomidae, Largidae, and Coreidae) (Dietrick and Van den Bosch, 1957).

Similarly, species of *Metaphycus* are all internal parasitoids of soft scales (Coccidae), but some are monophagous and others polyphagous. For example, in Australia, *Metaphycus maculipennis* (Timberlake) attacks only the grapevine scale, *Parthenolecanium persicae* (Fabricius), while *Metaphycus helvolus* (Compere) attacks a range of species of soft scales in at least three genera (e.g., *Ceroplastes destructor* Newstead, *Coccus hesperidum* L., and *Saisettia oleae* [Bernard]). In the same manner, aphelinids in the genus *Aphytis* are all ectoparasitoids of armored scales; but while several species are restricted to single host species, others have broad host ranges. *Aphytis lepidosaphes* Compere is specific to *Lepidosaphes beckii* (Newman) and *Lepidosaphes gloverii* (Packard), whereas *Aphytis diaspidis* Howard is polyphagous and parasitizes at least 50 species of armoured scales in 27 genera (Rosen, 1994).

This variation in host range, at species level, applies equally to predators. For example, the North American lacewing *Chrysopa quadripunctata* Burmeister is a generalist predator, whereas the very closely related and sympatric *Chrysopa slossonae* Banks is monophagous, preying only on the alder wooly aphid, *Prociphilus tendatus* (Fitch) (New, 1991). Coccinellids in the genus *Rodolia* are all predatory on fluted scales (Margarodidae), but the individual species may differ considerably in the breadth of their host range. For example, in Australia, the larvae of the native coccinellid *Rodolia cardinalis* Mulsant are known to prey on *Icerya purchasii* Maskell, *Icerya aegyptiaca* (Douglas), *Icerya seychellarum* (Westwood), and *Monophlebulus comperei* Morrison and Morrison, but *Rodolia koebelei* (Horn) has only been found preying on *Icerya koebelei* Maskell. Larvae of *R. koebelei* could only be reared to adults on *I. koebelei* and when they were offered *I. purchasii, I. aegyptiaca,* or *I. seychellarum,* they failed to feed and all died (V. Brancatini, pers. comm.).

USING INFORMATION ON AN AGENT'S BIOLOGY TO UNDERSTAND ITS HOST'S NICHE

Even literature that, strictly speaking, is not about host records can be useful in understanding an agent's likely host range. By understanding the exact host stages that a parasitoid requires for oviposition, researchers can better design host range tests. Information on seasonal cycles, on parasitoid behavior during host attack, or on host responses (such as encapsulation) can all be valuable in understanding the parasitoid's likely host range. Parasitoid host ranges are likely to be narrower if hosts occupy specialized habitats that require specific adaptations for successful exploitation. For example, the parasitoid *Cephalonomia stephanoderis* Betrem, a potential agent for the coffee berry borer, *Hypothenemus hampei* (Ferrari), is adapted to living in tunnels produced by its host in the berries of the coffee plant (Waterhouse and Norris, 1989; Waterhouse, 1998). The morphology of parasitoids and predators may also give some indication of their adaptive specialization and host preferences. For example, the white secretions produced by larvae of the Australian coccinellid *Cryptolaemus montrouzieri* Mulsant are very similar to the white secretions produced by many species of mealybugs, the predator's preferred prey.

Finally, all other things being equal, koinobiont parasitoids (species developing inside living hosts with immune systems, such as internal parasitoids of larvae, nymphs, or adults) are thought likely to be more host specific than idiobionts, which are either external to their hosts or are internal in eggs (which lack immune systems) (Godfray, 1994). Parasitoids that must contend with physiological host defences require a series of adaptations to defeat host immune responses and these requirements often limit the host range.

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CHAPTER 4. ANALYSIS OF FAUNA IN THE RECEIVING AREA FOR THE PURPOSE OF IDENTIFYING NATIVE SPECIES THAT EXOTIC NATURAL ENEMIES MAY POTENTIALLY ATTACK

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GOAL

The deliberate introduction and establishment of exotic natural enemies in a new geographic region to control a target pest as part of a classical biological control program entails some level of risk. One aspect of risk pertains to the potential adverse effect released natural enemies could have on desirable native and exotic fauna that are resident in the proposed region of introduction. To mitigate risk associated with classical biological control programs, the threat to the resident fauna needs to be assessed before new species are introduced. Major assumptions with such risk assessments are that the resident fauna is well studied from the phylogenetic, taxonomic, ecological, and biological view points and that the autecology of the natural enemy is known. Assuming a sound knowledge base exists, literature surveys, museum records, and biodiversity census data can be very useful in developing a list of potential organisms that could be at risk in the introduced range of the natural enemy. Further, information on the ecological requirements for successful development and population growth, and phenology of natural enemies in their home range can be used to further assess risk to non-targets in the introduced range by determining the level of overlap of key biological aspects between natural enemies and non-targets.

In many instances robust data sets will not be available for analysis, and the pest, its associated natural enemies, and the fauna potentially at risk may be entities new to science, further complicating such risk assessments. Besides obvious scientific impediments, political, legal, religious, and social doctrines need consideration when analyzing faunal risks. This is especially pertinent given the fluidity of prevailing social views and the propensity for them to change over time.

The goal of this chapter is to identify potentially important points that warrant consideration for determining what faunal components could be at risk from unintended attack when introductions of novel biological control agents into new areas are being undertaken, and what factors (scientific and social) exist that might facilitate or mitigate risk.

Some aspects of risk assessment using the existing literature or from laboratory assays have been covered in Chapters 3 and 7, respectively. Topics considered here include (1) determining the possibility of direct attacks on non-targets, (2) indirect effects, such as the implications arising from the creation of new food web linkages in the system under management, (3) the role of ecological and geographical filters in separating natural enemies from non-targets, (4) identifying organisms of special interest (i.e., rare, endangered, or unique native organisms or existing biological control agents), and (5) using existing infrastructures for guiding decision-making processes.

THE SCOPE OF NATURAL ENEMY IMPACTS

An important first step in determining the risk exotic natural enemies pose to the resident fauna in the new range of introduction is to develop a list of species that are potentially at risk. Ideally, the list of fauna at risk in the receiving area should be derived by exclusion and could be developed from phylogeny, morphology, physiology, behavior, geography, phenology, vagility, climatic requirements, and habitat preferences/fidelity. Risk associated with non-target impacts may be ecologically simple and result from direct attacks on non-target organisms. Alternatively, the risk to the receiving fauna may be complicated, arising from the development of unforeseen reticulated food web linkages mediated by competition (e.g., competitive exclusion), or food web subsidies (i.e., resource spill over of high density but ineffective natural enemies into other food chains), or by food web taxation (i.e., elimination/reduction/displacement of upper trophic level organisms from other food chains). Collectively, these effects can be referred to as indirect effects on non-targets caused by exotic natural enemies.

DIRECT ATTACK (TROPHIC IMPACTS)

Natural enemies that exhibit high levels of host and habitat fidelity ensure strong links and maximal impact on the target, while ensuring weak links to and minimal impacts on non-target species. When biological control projects stray from this fundamental ecological principle of high host specificity or the technology is applied without ecological justification to poorly chosen pest targets (e.g., neoclassical biological control attempts to utilize exotic natural enemies to suppress native pest populations [see Hokkanen and Pimental, 1989; Lockwood, 1993]), then undesired outcomes such as non-target impacts and lack of control are more likely to occur. Generalist natural enemies, by definition, lack high levels of host and habitat specificity. Such species are more likely to have adverse effects on native organisms and are less likely to control the target pest (Howarth, 1983, 1991; Simberloff and Stiling, 1996; Boettner *et al.*, 2000; Stiling and Simberloff, 2000; Henneman and Memmott, 2001). Database analyses indicate that pronounced non-target population changes by deliberately released arthropod biological control agents are infrequent. However, fewer than 2% of projects have data regarding the realized field specificity of released agents. This result is due, in part, to a lack of carefully planned studies that have sought specifically to quantify the effect natural enemies have on non-target organisms. This short-coming needs to be addressed to ensure that non-target organisms are not at undue risk and that legislation governing biological control introductions promotes responsible projects (Lynch *et al.*, 2001). While the exact ecological impact by biological control agents on native invertebrate populations is often uncertain, detailed studies using trophic spectra analyses (i.e., food webs) could be a powerful way to determine natural enemy impacts on the communities into which they are introduced (Memmott, 2000; Henneman and Memmott, 2001; Strong and Pemberton, 2001). Food web analyses focusing on exotic natural enemies used for biological control could provide profitable new research ground and would certainly assist in improving our understanding of how biological control agents interact with the receiving fauna in their new home range and help enhance prediction accuracy concerning the risk introductions pose to inhabitants in the natural enemy's new home range.

In an insightful retrospective study, Hawkins *et al.*, (1999) analyzed 68 lifetable studies of native insects and introduced insect pests to determine if biological control is analogous to naturally occurring control (i.e., the action of native natural enemies on native hosts). Hawkins *et al.* (1999) showed that successful biological control programs result in less reticulate trophic relationships than those seen in natural food webs of native insects. The most successful biological control programs do not have "natural" food web structures but rather consist of short linear food chains with less complex branching. This result occurs because biological control systems often consist of exotic species that share few ecological or evolutionary links with native biota. Furthermore, control is enhanced in simplified habitats that are characteristic of agro-ecosystems, and arguably, native systems that have been invaded by exotic plants, as both often consist of vast monotypic stands of exotic vegetation.

In summary, the available published data strongly suggests that direct attacks on non-target organisms by introduced natural enemies can be minimized by selecting agents with high levels of host and habitat fidelity. Such species are more likely to have a strong negative impact on the target, which as a consequence also drives down the population of the natural enemy as the host population contracts. This strategy holds ecological merit as it emphasizes interaction strength and is parsimonious as it reduces redundancy by avoiding the introduction of ineffective agents as part of guild reconstruction. The establishment of polyphagous natural enemies can adversely affect non-target populations, infiltrate habitats in which they are not wanted, and establish unwanted linkages into food webs which may manifest themselves as a major source for unwanted perturbations.

INDIRECT IMPACTS (FOOD WEB EFFECTS ON COMPETITION)

Adverse effects to non-targets not resulting from direct attack (i.e., indirect effects) are harder to anticipate than direct attacks and predicting indirect impacts requires greater knowledge of ecosystem functioning and a sound understanding of the historical range, abundances, and phenological variation of the non-target species of interest (Schellhorn *et al.*, 2002). The use of community modules in theoretical ecology studies has simplified to some extent the complexity associated with understanding ecosystem functioning and factors affecting key operational components (Holt and Hochberg, 2001). Theoretical studies have suggested several key issues that are likely to influence the severity of indirect impacts on non-target species: (1) risks to non-targets may occur from control agents that exhibit modest impact on the target; (2) highly vagile agents can invade ecosystems and influence food webs outside of release areas; and (3) resident natural enemies (i.e., primary or hyperparasitoids) that use the newly introduced natural enemy as a resource can themselves become a source of increased attack on non-target organisms (Holt and Hochberg, 2001).

Exotic natural enemy subsidization of food webs Exotic natural enemies that become superabundant in the environment because they fail to effectively regulate population densities of the target pest may become a resource that subsidizes the diet of native or exotic organisms, thereby affecting their population growth and interactions with other members of the community (Pearson and Callaway, 2003). For example, an ineffective weed biological control agent, *Urophora affinis* (Frauenfeld), released for the control of spotted knapweed, *Centaurea maculosa* Lamarck, provides an abundant food source for deer mice, *Peromyscus maniculatus* (Wagner). This subsidy has resulted in increased overwintering survivorship of mice, and high mouse numbers may affect populations of predators that use mice for food and promote increased disease transmission by mice (Pearson and Callaway, 2003). Similar results have been predicted for ineffective parasitoids that maintain high numbers on the target pest without regulating its population growth, thereby allowing large populations of exotic natural enemies to percolate into ecosystems where they could attack non-target organisms (Holt and Hochberg, 2001). Human mediated disturbances (e.g., regular harvesting) of agro-ecosystems can allow competitively inferior exotic natural enemies to outcompete native parasitoids that are superior in stable cropping systems, potentially allowing high numbers of exotics to spill out into surrounding environments to compete with native natural enemies in undisturbed habitats (Schellhorn *et al.*, 2002).

Food web taxation Natural enemies that effectively exploit non-target organisms in their new home range via direct attack may displace or eliminate resident native upper trophic level organisms that utilize the non-target as a primary food source. Deliberate or accidental introductions of competitive upper trophic level organisms may threaten rare native parasitoid species with extinction (Hochberg, 2000). Adverse effects on native hymenopterous parasitoids have almost certainly occurred with the establishment of *Compsilura concinnata* (Meigen) (Diptera: Tachindae) in the northeastern U.S.A. for control of 13 different pest species, including brown tail moth (*Euproctis chrysorrhoea* [L.]) and gypsy moth (*Lymantria dispar* [L.]), two serious forest pests. *Compsilura concinnata* is a polyphagous natural enemy that can utilize around 180 different species of Lepidoptera, Coleoptera, and Symphyta in North America (Boettner *et al.*, 2000). Boettner *et al.* (2000) have postulated that *C. concinnata* is primarily responsible for the regional declines of native saturniid moth populations in the northeastern U.S.A., and depending on the species and life stage, 36-100% of larvae may be parasitized by this fly in natural areas. Consequently, this natural enemy may have taxed the natural food web by displacing or removing key natural enemies that were essential components of the trophic structure associated with native saturniid moths (G. Boettner, pers. comm. 2004). A similar result has been observed in New Zealand, where *Trigonospila brevifacies* (Hardy) (Diptera: Tachinidae) was released for the control of the exotic tortricid pest *Epiphyas postvittana* Walker. This exotic natural enemy is the most abundant parasitoid attacking native tortricids in New Zealand's broadleaf and podocarp forests (Munro and Henderson, 2002).

Many coccinellids are neither prey nor habitat specific and may affect invertebrate biodiversity and even, perhaps, disrupt existing biological control programs (Obrycki *et al.*, 2000). For example, *Coccinella septempunctata* L., an exotic coccinellid introduced into North America for aphid control, has influenced the distribution and abundance of native coccinellid competitors by reducing their survivorship in local habitats, influencing dispersal dynamics and habitat use. *Coccinella septempunctata* has displaced native coccinellids in agro-ecosystems by reducing prey abundance, as native species are more responsive to localized prey densities (Elliot *et al.*, 1996; Evans, 2004). Broad dietary breadth, propensity for intraguild predation, large size, and aggressive behavior can further facilitate displacement of native coccinellids from agro-ecosystems by exotic ladybirds (Michaud, 2002). Disruptive effects by coccinellids in non-agricultural habitats have not been documented, but laboratory feeding studies using native species as prey suggest that they could occur (Obrycki *et al.*, 2000).

Apparent competition Apparent competition occurs when an abundant host causes an increase in the population density of a food-limited natural enemy that exploits that host as resource. This results in population growth of the natural enemy and greater attack rates on the focal host and any alternate hosts the biological control agent uses as food (Holt and Lawton, 1994). Mechanisms behind apparent competition can be varied, and include cases in which attack rates are greater on one species than the other and cases in which attack rates are similar. Declines of a non-target species because of apparent competition could result when attack rates on the non-target species are elevated by a preference for that prey by the natural enemy. Alternatively, if attack rates are similar, population declines could occur because the fecundity of one host is lower than the other and that species is unable to absorb the additional mortality. Consequently, natural enemies with overlapping host ranges may change the diversity of host assemblages. The decline of the native *Pieris napi oleracea* Harris in parts of New England (Massachusetts, principally) where it is sympatric with the exotic and pestiferous *Pieris rapae* L. is thought to have occurred, in part, because of apparent competition resulting from the introduction of the braconid parasitoid *Cotesia glomerata* (L.) (Benson *et al.*, 2003). Apparent competition may have affected the abundance of rare host specific native parasitoid species associated with native tortricids in New Zealand following the introduction of *T. brevifacies* (Munro and Henderson, 2002).

Ecological replacement Causative links in ecological networks are often unseen and hard to trace because effects can be very indirect. Changes in the behavior or abundance of one species can have far-reaching effects on an apparently unrelated species, affecting its ability to survive. In some instances, a pest species may have become an integral part of ecosystem functioning and a variety of native species utilize the exotic pest as a resource. Successful biological control of such an essential resource may imperil native species that rely on it. For example, biological control of rabbits (an introduced species) with the myxoma virus in Great Britain was one of several inter-related factors that resulted in the extirpation over large areas of an endemic and endangered species, the large blue butterfly, *Maculinea arion* (L.) (Lepidoptera: Lycaenidae). This butterfly requires nests of the ant *Myrmica sabuleti* Meinert for larval development and the ant depends on a species of grass that preferential rabbit grazing allowed to proliferate. The decline of rabbit populations because of myxomatosis resulted in a subordinate grass species rising to dominance, which adversely affected ant nesting success and ultimately the breeding success of *M. arion* (Ehler, 2000).

In New Zealand, the endangered Mahoenui giant weta, *Deinacrida* sp., (Orthoptera : Anostostomatidae) was discovered inhabiting a large infestation of gorse, *Ulex europaeus* L., in the central North Island. This weta was a species new to science at time of discovery, and the inhospitable spines on gorse allowed weta populations to escape rat predation in dense thickets. The gorse in which the weta lives has been set aside as a preserve (Meads, 1990), and yet, more broadly, gorse is currently the target of a major classical biological control program in New Zealand.

The endangered southwestern willow flycatcher (*Empidonax trailii extimus*) nests extensively in salt cedar (*Tamarix* spp.) in the southwestern U.S.A. This alien weed is the subject of a classical biological control program, and there is concern that successful control of this invasive tree will further reduce nesting habitat available to flycatchers (DeLoach *et al.*, 2000) unless salt cedar reduction is slow and concurrent with regrowth of stands of cottonwood trees, which is the native species originally used for nesting.

Disruption of existing biological control programs Development of trophic relationships among introduced and native biological control agents can interfere with the successful establishment, spread, and impact on the target pest of newly introduced natural enemies. Polyphagous coccinellids can disrupt low density pest regulation by parasitoids by consuming parasitized aphids, which prematurely removes parasitoid progeny from the system. Reductions of aphid populations by predation can also remove carbohydrate sources – such as honeydew – that parasitoids utilize as an energy source Loss of such foods can affect foraging efficacy, fecundity, and longevity, further disrupting control exerted by natural enemies on other pest species (Obrycki *et al.*, 2000). Feeding by coccinellids on infected aphids can reduce rates of disease transmission during fungal epizootics, and generalist ladybirds may affect population densities of herbivorous biological control agents by feeding on eggs and larvae (Obrycki and Kring 1998).

Tetranychus lintearius Dufour (Acari: Tetranychidae), released for the biological control of gorse in New Zealand, established widely and exhibited rapid population growth but was quickly suppressed by the endemic coccinellid *Stethorus bifidus* Kapur, which limited the mite's impact on the target weed. In Oregon (U.S.A.), *T. lintearius* has acquired a guild of specialist and generalist phytoseiid mites that have been routinely used for biological control of pestiferous tetranychids in agricultural systems. The key predator attacking *T. lintearius* on gorse in Oregon appears to be *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) (Pratt *et al.*, 2003).

Gall-forming tephritids introduced into Hawaii for the biological control of weed species have attracted guilds of exotic opiine braconids that have been introduced for the biological control of pest tephritids attacking fruit (Duan and Messing, 2000). Future introductions of opiine fruit fly larval parasitoids against frugivorous tephritid pests should consider potential impacts on such gall-forming tepritids, so as to protect biological weed control agents.

The importance of ecologically simple (direct attack) or complicated (unintended, indirect, reticulate) food web linkages may be impossible to estimate prior to releases of natural enemies. Indeed, such effects may not be detectable for many years after releases are made, even assuming that the system of interest is studied specifically for assessing the magnitude of such non-target impacts. However, combinations of critical factors (e.g., phylogenetic relatedness of native species to the target pest, overlap in their habitat use, geographic ranges, or climatic requirements) could be used to delineate those native species most likely to be at risk.

For those organisms identified as being at potential risk (i.e., native species and deliberately introduced exotic species such as other biological control agents), protective priorities need to be determined. Preservation efforts might attempt to protect all non-target organisms from natural enemy attack. Or, it may be more practical to focus on ones that are rare, beneficial, or beautiful, or that act as keystone (i.e., organisms with disproportionately large community effects relative to their abundance) or flagship species (i.e., ones that serve as symbols and rallying points to stimulate conservation awareness and action). Should risk to specific non-targets be identified, consensus amongst stakeholders (i.e., biological control proponents, conservationists, ecologists, lay public, and indigenous peoples' representatives) is needed to determine what level of risk posed by the natural enemy – none (no non-targets are attacked), low (few individuals attacked), medium (some localized population suppression occurs), or high (population suppression is sufficient to cause range contraction over a large spatio-temporal scale) – is acceptable.

PHYLOGENETIC RELATEDNESS AND SPECIFICITY: THE PLANT BIOLOGICAL CONTROL EXAMPLE

The degree of relatedness of non-target organisms to the target pest may be indicative of the likelihood of attack by introduced natural enemies. A comprehensive study by Pemberton (2000) assessing non-target attacks on native plants by exotic weed biological control agents clearly indicated that the more closely related the non-target species was to the pest, the more likely it was to be attacked. Safe targets for biological control had few or no native congeners, and likelihood of attack declined significantly with decreasing relatedness. The centrifugal-phylogenetic approach (Wapshere, 1974) for choosing which non-target plant species to include in schemes for host specificity testing has been very successful for predicting and limiting non-target impacts on plants. In part, this system works because plants are less speciose than herbivorous insects and a much higher proportion of them have been described scientifically. For many groups, plant phylogenetic relationships are well known, which is usually not so for arthropods.

The moth *Cactoblastis cactorum* (Bergroth), a native of Argentina, is a classic example of successful weed biological control, famous for suppression of weedy cacti in Australia (Stiling, 2002). A less desirable outcome occurred when this moth was released on the island of Nevis in 1956 and subsequently spread naturally to the continental United States (in 1987), where it now attacks some common and endangered native North American cacti (Pemberton, 1995).

Cactoblastis cactorum is a "specialist" on the cactus genus *Opuntia*, a group with approximately 200 species in the Americas (Mahr, 2001). On continents such as Australia that lack native cacti (where it was released in 1926 for control of weedy *Opuntia* spp.) *C. cactorum* has adequate specificity to protect native plants. By contrast, in North America, *C. cactorum* is better seen as a generalist invader, as it is not specific to any one species of *Opuntia* and can feed and reproduce on a large number of species in the genus, threatening many rare and endangered cacti (Stiling 2002). This example illustrates that "how specific is specific enough?" is context dependent.

Two thistle-feeding insects, *Rhinocyllus conicus* (Frölich) (intentionally released, Gassmann and Louda [2001]) and *Larinus planus* (Fabricius) (an accidental arrival in the United States that had been eliminated as a potential biological control agent because of broad host breadth on Carduinae thistles in its home range [Louda and O'Brien, 2002]) attack several native North American thistles. This was anticipated from host specificity tests as both weevils were known to feed and reproduce on a variety of thistle species in their home and introduced range. Both insects are "specialists" in the sense that they feed only on thistles, but thistles are a speciose group with many representatives in several genera that occur both in Europe and North America. The broad dietary breadth of these weevils among thistles (species in three genera) makes them "thistle generalists" in Europe and North America. However, in countries such as New Zealand, which lacks a native thistle flora, *R. conicus* is sufficiently host specific for use as a biological control agent as it does not feed outside of the thistle group, making it a true "specialist" relative to the plants of New Zealand.

In both of the preceding examples, the risk to the resident flora was affected by the taxonomic relatedness of the target weed to locally present native plants and by the host breadth of the natural enemy (i.e., family, tribe, or species level of host specificity).

BEYOND THE CENTRIFUGAL PARADIGM: PREDICTING THE IMPACT OF ENTOMOPHAGOUS INSECTS

Assessments of the risk exotic arthropods, in particular parasitoids, pose to native fauna using the centrifugal-phylogenetic strategy used in weed biological control host specificity assessments may not be the best approach for determining which members of the non-target arthropod fauna in the receiving area will be at risk. One obstacle to using this approach for assessing risk to non-targets and natural enemies is uncertainty about phylogenies of nontarget arthropod groups, as many species are undescribed and relationships within even well studied groups often may lack consensus on lower- and higher-order associations (Messing 2001). In addition to the above "taxonomic impediment," the overwhelming numbers of arthropod species that could be tested (in comparison to plant species) creates a unique set of problems (Barratt *et al.*, 2000). Furthermore, the host utilization of many parasitoid species shows no clear taxonomic derivation, but rather is driven by type of habitat use or the feeding strategy of the host (e.g., leafmining guilds across several orders [Lepidoptera, Diptera, and Hymenoptera] often share common parasitoids). Interactions between the herbivore, its host plant, and their shared microhabitat can produce unique sets of interacting factors that strongly influence host selection and use (Messing, 2001).

Retrospective analyses of arthropod biological control programs have attempted to tease out general principles governing host use by entomophagous biological control agents. Approximately 16% of all introduced parasitoids attack some species of native insects (Hawkins and Marino, 1997). Among introduced parasitoids whose post release host ranges have been investigated, 11% failed to establish on the target pest and were recorded only from native hosts. It is possible that this non-target host use was temporary, or that parasitoids were misidentified, or that the native hosts were actually more preferred than the target pest (Hawkins and Marino, 1997). The likelihood that exotic parasitoids would attack non-targets was unpredictable with respect to analysis of six independent variables: (1) the parasitoid's biology, (2) the parasitoid's region of origin, (3) whether the parasitoid successfully established on the target and suppressed its population growth, (4) the feeding niche of the target, (5) habitat use by the target, and (6) the amount of time that had elapsed since introduction.

Hawkins and Marino (1997) concluded that the poor quality of the data sets they used for their retrospective analyses, the stochastic nature of ecological systems, and an imperfect understanding of factors affecting host range determination in parasitoids made it impossible for them to accurately predict the risk exotic parasitoids posed to non-target organisms in the receiving area.

The risks posed by introduced predators to non-target species may be significantly greater as predators are often less host specific than parasitoids (Hawkins and Marino, 1997). The magnitude of any attacks on non-target hosts and consequent effects on ecosystem functioning are largely undetermined (Hawkins and Marino, 1997), but generalist parasitoids with broad host and ecological ranges have been implicated in declines of some native insects (Boettner *et al.*, 2000).

Even in instances where natural enemies have been subjected to rigorous host specificity testing and the physiological host range has been accurately identified, the ecological host range and population-level impacts on less preferred but acceptable native species can not be accurately predicted as community wide interactions are complex and ecological risks can be difficult to identify and disentangle (Louda *et al.*, 2003a). Retrospective analyses of several well-studied biological control projects deliberately looking for non-target impacts by exotic natural enemies suggest the following trends may exist: (1) close relatives of the target are most likely to be attacked, (2) the level of impact on non-targets is varied and affected by environmental conditions, (3) non-target impacts can accelerate the decline of rare native organisms, and (4) native ecosystems can be invaded by natural enemies released for control of pests in areas intensively managed by humans (e.g., agro-ecosystems) (Louda *et al.*, 2003b). Retrospective analyses of exotic parasitoids released in Hawaii indicate that non-target impacts and habitat infiltration can be significantly reduced by selecting ichneumonid and braconid parasitoids with narrow host breadths and high levels of habitat fidelity (Henneman and Memmott, 2001).

Barratt *et al.* (1999) suggest that, within the constraint of regulatory requirements, the following ideas can be used to assess the risk to fauna in areas that could potentially receive arthropod natural enemies: (1) assess risk by testing phylogenetically/taxonomically species closely related to the target; (2) examine ecological affinities between the target pest and native fauna by identifying non-target species that occupy similar niches the proposed natural enemy could exploit; (3) determine if non-target impacts have occurred in other areas where the natural enemy has been employed; (4) use key findings from steps one to three above to develop a non-target list that could be subjected to laboratory host specificity tests. Further refine the list of non-target species once initial laboratory data are analyzed.

For example, the risk of the 1971 introduction of the braconid *C. glomerata* to native pierid butterflies in Chile and Argentina may have been predictable on the basis of the habitat and host plants of pierids, plus subfamily level taxonomic relatedness to the usual hosts of this parasitoid. Specifically, we would have predicted that species of Pierinae (whites) are at high risk, while sulfurs (Coliadinae) are at low risk, and orange tips (Anthocarinae) are at intermediate risk, based on known hosts from the literature. Within the Pierinae, species that feed on mustard oil plants (the typical food plant group) would be at greater risk than ones that feed on legumes. Also, species found at low elevations in agricultural and suburban habitats (the habitat of the target host, *Pieris brassicae* L.) would be at high risk, while those found at high altitude on cushion plants would be at little to no risk (depending on what turns out to be the upper altitudinal limit of *C. glomerata*, which is believed to be around 8000 feet). With this perspective, one could move to laboratory host range testing, coupled with field surveys (since the release has already been made) to verify these predictions.

In some instances, the risk to the non-target fauna in the area receiving exotic natural enemies of arthropods may be very obvious and adverse effects can be foreseen. For example, the diversity of drosophilids in the Hawaiian islands is a textbook example of species radiation in an insular island ecosystem. Consideration of releases of exotic eucoilid parasitoids for control of pestiferous exotic drosophilids would be ill advised because of the high likelihood of non-target attacks, the difficulty and expense associated with surveying the native drosophilid fauna, accurately identifying cryptic species using behavioral, morphological, and molecular techniques, and conducting host specificity testing of native Hawaiian drosophilids. Similarly, New Zealand tortricids have a very high level of endemism. Of the 185 described New Zealand species, 174 are native to New Zealand (Munro and Henderson, 2002). To safeguard this unique fauna, natural enemies considered for the importation and control of exotic pestiferous tortricids of fruit crops must exhibit extremely high levels of host and habitat fidelity and be likely to exert strong population suppression on the target pest.

LESSONS FROM INVASION BIOLOGY

Natural enemy introductions are planned invasions in which exotic agents are deliberately introduced into a new area and factors affecting their establishment, spread and impact are promoted. Prediction of potential risks to the non-target fauna may benefit from recent advances in emerging theory from the field of invasion biology. The opportunities provided by the invaded community will strongly influence the interactions of the natural enemy and various non-target organisms. Two important factors that can facilitate invasion are resource abundance and niche availability.

Resource opportunities can affect invasion success when resources are high either because they are abundant or because native natural enemies do not interfere with access to the resource so that there is opportunity for exploitation by invaders (e.g., exotic natural enemies) with a proclivity to do so (Holway and Suarez, 1999). When resources are contested, invaders with higher rates of resource acquisition, lower energy demands, or higher intrinsic rates of increase can displace native species from that resource base (Shea and Chesson, 2002). The invader may not necessarily be superior in all aspects to native competitors, but may have a superior response to a particular resource or the temporal/spatial availability of that resource, especially when native residents do not keep that resource at uniformly low levels over time (Chesson, 2000).

Niche availability can facilitate invasion success. The empty niche hypothesis predicts resource exploitation by an invader will occur when species diversity is low (e.g., on islands) because the resource is not being exploited efficiently due to a lack of local species with suitable niche adaptations (Simberloff, 1995). In contrast, under conditions of locally high species diversity, invasion success should depend not on filling a vacant niche (which presumably are all filled), but on being a better exploiter of resources or a better avoider of local natural enemies than the species previously using the resource (Chesson, 2000).

Combinations of these processes (resource and niche availability) may allow exotic natural enemies to invade unintended areas and attack non-target organisms in ways that can be difficult to predict from laboratory host range studies.

CLIMATIC REQUIREMENTS AND GEOGRAPHIC OVERLAP

A fundamental requirement for the establishment of any species outside of its home range is that the recipient location must have a climate similar to the invader's area of origin. Assuming that climate is a major factor affecting establishment success or failure and that it influences the likelihood of invasion from the introduced range, matching the climate of the home range to potential recipient regions can be used to determine the suitability of areas under invasion risk and subsequent invader spread to vulnerable regions. Consequently, if it can be demonstrated that a natural enemy is unlikely to extend its range and establish transient populations in areas with potential non-target hosts, then the risk to that fauna is predicted to be reduced.

Climate matching methods range from simple indices that allow graphical comparisons across localities to computer software that match climates and relate species distributions or ecophysiological responses to environmental variables (Worner, 2002). CLIMEX is one such climate matching computer program. This is a predictive tool that can be used to ascertain an organism's potential abundance and distribution using biological data and observations on its known geographical ranges (Sutherst and Maywald, 1985). CLIMEX has been used to determine the potential distribution of natural enemies following release in new locales (Mo *et al.*, 2000), assessing invasion risk posed by exotic cerambycid beetles (MacLeod *et al.*, 2002), determining the potential geographic distribution of economically important tephritid flies (Vera *et al.*, 2002), and elucidating climatic factors limiting the distribution of pestiferous soil mites (Robinson and Hoffmann, 2002). Similar applications could be used for determining the likelihood of natural enemy spread beyond the intended release area. However, quality of weather data sets will affect model predictions. For example, such models failed to predict the establishment of the vedalia beetle, *Rodolia cardinalis* Mulsant, in the Galápagos, yet this natural enemy did establish, an outcome Causton (in this volume) attributed to poor information on rainfall.

SEEKING ASSISTANCE IN CONSTRUCTING CANDIDATE POOLS

Determining what fauna is at risk in the receiving area and accurate identification of candidate natural enemies proposed for release will significantly affect the utility of risk assessment lists. Lists of taxonomists important to biological control are available (*http://www.cnr.berkeley.edu/biocon/id_insects/taxlist.htm*), and employment of a taxonomist skilled in morphological and molecular techniques may be crucial in the initial stages of developing a risk assessment agenda, especially when the fauna being examined is poorly known. Specific requests for information or assistance can be made over internet news groups such as BIOCONTROL-L, PARAHYM, ALIENS-L, and THRIPSNET.

LEGISLATIVE GUIDELINES AND VOLUNTARY CONDUCT CODES

Assessment of risk to receiving fauna can be assisted to some extent by utilizing existing voluntary guidelines or legislation adopted by countries that strictly regulate the importation and release of exotic natural enemies. For example, the adverse effects arising from migratory species, such as *C. cactorum,* could be reduced by assessing potential ecological risks associated with natural enemy dispersal. The Technical Advisory Group (TAG) consists of representatives from the U.S.A., Canada, and Mexico who assess risk posed by proposed weed biological control agents and their propensity to cross international borders to threaten non-targets (CoFrancesco, 1998). However, the authority of TAG does not extend into the Caribbean (and therefore could not have influenced the release of *C. cactorum* in this region) or beyond Mexico's southern border with Guatemala. In these instances, regional bodies should be established to communicate and assess risk about intended natural enemy releases. For example, a consortium of countries with interest in natural enemy releases in the Caribbean would not only include Caribbean nations, but could also involve Florida (U.S.A.), Mexico, Central America and northern South America. Similar consortia that mediate consultation could be useful for planned natural enemy releases on islands in the South Pacific Ocean and could be mediated by the South Pacific Applied Geoscience Commission (SOPAC) based in Fiji. Member countries of SOPAC include Australia, Cook Islands, Federated States of Micronesia, Fiji Islands, Guam, Kiribati, Marshall Islands, Nauru, New Zealand, Niue, Palau, Papua New Guinea, Samoa, Solomon Islands, Tonga, Tuvalu and Vanuatu, American Samoa, French Polynesia, and New Caledonia.

The Food and Agricultural Organization of the United Nations (FAO, 1997) and North American Plant Protection Organization (NAPPO, 2000, 2001) encompasses Canada, the U.S.A., and Mexico. Both provide guidelines to assess risk posed by entomophagous and phytophagous natural enemy movement across international borders. Several countries have developed new or revised existing legislation to minimize environmental risks and non-target impacts associated with importing and releasing exotic natural enemies (COSAVE 2004; ERMA 2004). These legislative requirements provide guidelines to ascertain risk to non-target organisms that could be adversely exposed to exotic natural enemies. ERMA (2004) requires consultation with Iwi (i.e., the indigenous people of New Zealand, the Maori) as part of the decision-making process when assessing the spiritual importance of indigenous flora and fauna and any potential risks they may be exposed to. This is a mandatory requirement for assessing the risk to native organisms when applications for deliberately introducing new organisms into New Zealand are made (Barratt et al., 2000).

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CHAPTER 5. BEHAVIORAL AND PHYSIOLOGICAL PROCESSES AFFECTING OUTCOMES OF HOST RANGE TESTING

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INTRODUCTION

Estimation of the host range of entomophagous biological control agents (parasitoids and predators) is more complex than for phytophagous weed biological control agents. This is primarily because there is an additional trophic layer involved and often an intimate and specific relationship between the target and test organisms and their substrate (usually their food plant). An important consequence of this intimate and specific relationship between the host or prey of entomophagous agents and their substrate is that prior experience of the substrate can affect the organism's responsiveness to cues from this and other substrates.

A second complicating factor for endoparasitoids is that it is not possible, in most cases (although exceptions do exist: Morehead and Feener, 2000; Fuester *et al.*, 2001), to inoculate all test organisms with eggs or neonates to determine "suitability." Thus, a program to determine the host range of parasitoids is denied one of the most powerful tools (the so-called physiological host range test) used in determination of host range of phytophagous agents (Hill, 1999; van Klinken, 2000). This means that, in the host range testing of parasitoids, it is important to employ test procedures that will maximize the probability that the test species will be accepted for oviposition. This is vital for an accurate risk assessment.

In this chapter, we discuss the effects of experience and physiological state on the host range expressed by parasitoids and predators. We suggest ways the potential agent might be treated before testing and the form the test should take to maximize the expression of host acceptance. It must be understood that effects of experience, deprivation, and age often are not clearly separable in any given circumstance. Nevertheless, we have taken the approach of discussing the known effects of each separately to illustrate the processes that might be involved.

INFLUENCE OF INFORMATIONAL AND PHYSIOLOGICAL STATES OF PARASITOIDS

Effects of Experience

Comprehensive reviews exist that thoroughly cover the phenomena of learning in hymenopterous parasitoids (Turlings *et al.*, 1993; Vet *et al.*, 1995). Thanks to the high quality of the literature now available, we have a good appreciation of the complexity of experience effects on host-related behavior in parasitoids.

The nature of experience-induced changes in responsiveness There are two key effects of experience that can influence the outcomes of various designs of host specificity tests. First, there is change in the responsiveness of females to a previously experienced (i.e., familiar) host and/or the substrate host complex (SHC). Second, there is any change in the responsiveness to an unfamiliar host or SHC.

Generally, responsiveness to a familiar host or SHC is enhanced by experience. This phenomenon has been observed in relation to the rearing host and to the rearing host's substrate. This phenomenon has also been demonstrated in relation to experience later in life of the complete SHC or some of its components, with or without oviposition.

Experience-induced changes in responsiveness have been demonstrated most unequivocally in no-choice assays comparing the behavior of naive and experienced females. A number of studies have shown that the probability of upwind flight is higher in experienced females than in naive females, and that the relevant experience may come from (1) the odors of a familiar host or SHC (e.g., Monge and Cortesoro, 1996; Hérard *et al.*, 1988), (2) effects of the rearing substrate (Monge and Cortesoro, 1996), (3) effects of host remains (Parra *et al.*, 1996; Du *et al.*, 1997), (4) contact with the SHC but without oviposition (Du *et al.*, 1997; Potting *et al.*, 1999; Daza-Bustamante *et al.*, 2002), or (5) the effect of oviposition experience on the SHC. Other studies have demonstrated an increased probability of host acceptance (e.g., Ambriz *et al.*, 1996; Bjorksten and Hoffman, 1998) or of probing (e.g., Kerguelen and Cardé, 1996) in response to a familiar host or to components of a familiar SHC in experienced females. Others, again, have shown that searching times on a familiar host or SHC is greater in experienced females than in naive ones (e.g., Iizuka and Takasu, 1998).

There is much less directly comparable information available in relation to experienced-induced changes in responsiveness to an unfamiliar host or SHC from no-choice assays. In theory, responsiveness to unfamiliar hosts or SHCs may be enhanced, unchanged, or reduced. Enhanced responsiveness could come about as a result of priming or sensitization (see Turlings *et al.* [1993] for discussion of this phenomenon). At least two instances of enhanced responsiveness to an unfamiliar SHC have been shown in no-choice assays (Turlings *et al.*, 1989; Eller *et al.*, 1992). We are aware of only one study that provides evidence for reduced responsiveness to unfamiliar hosts. Kitt and Keller (1998) showed that naive females of *Aphidius rosae* accepted several non-target species of aphid in no-choice tests, whereas females that had oviposition experience on the target species did not. This indicates that the possibility of reduction in responsiveness to unfamiliar hosts or SHCs by experienced parasitoids must be borne in mind when designing host testing protocols.

There is strong but indirect evidence that any enhancement in responsiveness to a familiar host or SHC is generally greater than any enhancement in responsiveness to an unfamiliar host or SHC. This comes from the result of choice assays in which the choices made by naive females have been compared with choices made by experienced females. Typically, the experienced females are more biased toward the familiar host than naive females. For example, naive females of *Cotesia kariyai* Watanabe strongly preferred the odor of host-infested corn plants over the odor of infested kidney bean plants (about 80% flight to infested corn). In contrast, females that had oviposited on hosts on kidney bean plants showed a preference for the odor of infested kidney bean (less than 40% of flight to infested corn) (Fujiwara *et al.*, 2000). Comparable results have been obtained by Pettit *et al.* (1992) for the braconid *Opius dissitus* Muesebeck. Similar results with respect to host acceptance following experience during eclosion have also been obtained with *Aphytis melinus* DeBach (Hare, 1996).

A substantial proportion of studies of the effects of experience on the foraging behavior of parasitoids (and phytophagous insects) has employed the classical induction-of-preference paradigm. In this, the choices made between host (or SHC) "A" vs. "B" are determined in insects that have had prior experience of either "A" or "B". Frequently, it has been found that the choices made are relatively biased towards the familiar host or SHC (e.g., Petitt *et al.*, 1992; Geervliet *et al.*, 1998; Fujiwara *et al.*, 2000; Daza-Bustamante *et al.*, 2002). As pointed out by Bernays and Weiss (1996), results of this kind do not distinguish between enhanced responsiveness to the familiar host (or SHC) and reduced responsiveness to the unfamiliar. Nevertheless, results from this type of experiment have often shown profound effects of experience. In one striking example of relevance to the use of choice tests, it was shown that females of the aphidiid parasitoid *Aphidius ervi* (Haliday) that have oviposited in the aphid *Acyrthosiphon pisum* (Harris) on alfalfa exclusively chose the odor of the familiar SHC over the odor of *Sitobion avenae* (F.) on wheat, but females with experience of *S. avenae* on wheat exclusively chose the odor of this SHC over the odor of *A. pisum* on alfalfa (Daza-Bustamante *et al.*, 2002).

Implications of experience-induced changes for host range testing It follows from the aforementioned articles that the general expectation is for a parasitoid to be biased toward a familiar host or SHC in host range testing programs. What this means in practical terms depends (i) upon the history of the parasitoids used in the tests, (ii) how the test organisms (target and non-target) are presented in the tests, (iii) the form of the test(s) (choice versus no-choice; see Chapter 7), and (iv) the magnitude and nature of the effects of previous experience. For example, one likely scenario is for a parasitoid to be reared on the target organism feeding on a particular host plant. The experience gained of the rearing host and its host plant during eclosion and perhaps during larval development is likely to result in enhanced responsiveness to cues from this SHC. It is likely that this would be reinforced by continued contact with, and possibly oviposition experience on, the rearing SHC, if the parasitoids were not removed from the rearing colony before or shortly after eclosion.

Therefore, if the parasitoid had a greater innate preference for a SHC consisting of the target species on its host plant compared with a SHC consisting of a non-target species on a different host plant, then experience of the target SHC would, in effect, exaggerate the apparent difference between the rankings of the two SHCs. This would have somewhat different implications for the interpretation of results from choice and no-choice tests, particularly when the target species and non-target species are presented on different hosts. In choice tests, increased contrast in ranking between the SHCs would, in itself, increase the probability that there would be no attack on the non-target species. In the case of no-choice tests, the results of tests could be influenced by any reduction or enhancement in responsiveness to unfamiliar, non-target SHC caused by experience of the target SHC. If responsiveness to the unfamiliar SHC were reduced, this would also decrease the probability of attack on the non-target species. On the other hand, if responsiveness to the unfamiliar, non-target SHC were enhanced (e.g., by priming), this would increase the probability of attack on the non-target species. Both outcomes would be influenced by the duration of the no-choice assay compared to the duration for which the effect of experience remains.

Strategies that might minimize the undesired effects of experience There are ways that experience-induced bias towards the target species can be reduced or even eliminated. The most difficult effect to avoid is any enhanced responsiveness to the rearing host, unless high quality parasitoids can be reared on an alternate host. This is particularly useful if there is an influence of experience acquired during pre-imaginal development, a phenomenon that has very rarely been demonstrated either for a host species or its substrate (Gandolfi *et al.*, 2003). More commonly, it has been shown that the apparent influence of the larval host or its substrate has been the result of early adult experience acquired at eclosion or shortly afterwards (e.g., Hérard *et al.*, 1988; Monge and Cortesoro, 1996). For crucial tests in these circumstances, the effects of the rearing environment may be avoided or reduced by such methods as dissecting the parasitoid pupae out of the host and washing the pupae prior to eclosion.

Bias in favor of the target species' host plant can be avoided if it is possible to rear the target species on two or more hosts or on a synthetic diet. This opens the possibility of presenting the target species in tests on a different substrate from that which the parasitoids experienced during pre-imaginal stages and/or during eclosion. A more practical approach to avoiding a possible bias towards the host's substrate used in the rearing of the parasitoids may be to present target and non-target species to the parasitoid on an "inert" substrate such as glass. However, this is impossible wherever test species are inseparable from their hosts, such as with internally feeding larvae, scale insects, or mealybugs.

We recommend that any bias as a result of experience of the SHC, with or without oviposition, later in the life of the parasitoid could readily be avoided by collecting the parasitoids immediately at or soon after eclosion and storing them in the absence of hosts and plant material. As indicated above, whether avoidance of oviposition experience on the target species would be desired would depend on the type of test and the effect of such oviposition on responsiveness to unfamiliar non-target hosts or SHCs. We suggest that, if no-choice tests are employed, ideally they should be done both with female parasitoids that have had oviposition experience on the target species and also comparably with females that have been denied the opportunity to oviposit.

EFFECTS OF HOST DEPRIVATION

The responsiveness of female insects to cues associated with oviposition sites is known to be affected by host deprivation (Papaj and Rausher, 1983; Barton Browne and Withers, 2002). The general expectation is that there will be a positive correlation between readiness to oviposit and elapsed time since the female last oviposited or since she emerged. The most important practical result of this is that lower ranked hosts are more likely to be accepted as the period of deprivation increases.

Most of the evidence for this in parasitoids comes from the finding that host-deprived females (e.g., Klomp *et al.*, 1980; Hubbard *et al.*, 1999) and/or females that have had low encounter frequency with unparasitized hosts (e.g., Babendreier and Hoffmeister, 2002) show increased acceptance of hosts already parasitized by conspecific females. In the species studied, females were able to rank parasitized hosts lower than pristine hosts because of the presence of host-marking pheromones and/or because of internal changes induced within the host (see review in Nufio and Papaj, 2001). However, there is evidence that the probability of accepting lower ranked host species also increases with the period of host deprivation. Host-deprived females of the chalcidiid *Brachymeria intermedia* (Nees) showed a 30% probability of acceptance of a higher ranked host, the pupae of the gypsy moth, *Lymantria dispar* (L.), about three days after eclosion. The same rate of acceptance of a lower ranked host, pupae of *Holomelina lamae* Freeman, was displayed only after about 10 days, by which time the acceptance rate of *L. dispar* pupae had increased to more than 70% (Drost and Cardé, 1992).

Life history theory predicts that readiness to oviposit would be influenced by egg load and/or host encounter rate during some period immediately preceding the current encounter (Mangel, 1989). Empirical data for parasitoids and other insects support this prediction (e.g., Minkenberg *et al.*, 1992; Hughes *et al.*, 1994; Babendreier and Hoffmeister, 2002). Host deprivation always has the potential to give the insect a low expectation of encountering hosts and, hence, an increased probability of accepting lower ranked hosts. However, the effect of host-deprivation on the egg load, and therefore the potential contribution of this factor to any increased readiness to oviposit, is dependent on ovarian physiology. For example, a female of a pro-ovigenic species, by definition, will not increase its egg load over a period of host deprivation, and any increase in readiness to oviposit in pro-ovigenic species cannot be attributed to this factor. On the other hand, there is a potential for females of synovigenic species to increase their egg load, at least up to point, during a period of host deprivation (e.g., Eliopoulos *et al.*, 2003). The extent to which this happens, if at all, would be dependent on the nutritional reserve stored within the body and/or the availability of suitable foods during the period of host deprivation. This is particularly relevant in host-feeding species when host deprivation deprives the females of nutrients for oogenesis as well as depriving them of the opportunity to oviposit. For example, when the host-feeding species, *A. melinus*, is maintained on honey but deprived of hosts, there is a reduction in egg load due to oosorption (Collier, 1995). Reduction in egg load has also been suggested as a probable explanation for the decline in readiness to oviposit seen in *B. intermedia* (Drost and Cardé, 1992) after a prolonged period of host deprivation.

Host deprivation could be generally expected to increase the probability of acceptance of lower ranked hosts except, perhaps, when host deprivation results in a reduction in egg load, as is likely in host-feeding species. It is relevant to note, however, that the egg load in *A. melinus* did not decrease in females maintained on yeast in addition to sucrose (Heimpel and Rosenheim, 1995). Thus, any reduction in egg load during host deprivation in host-feeding species might be avoided or minimized by the provision of a suitable nitrogen-containing food in addition to a source of carbohydrate.

AGE AND LIFE EXPECTANCY

We discuss the effects of age and life expectancy in terms of "ovigeny" characteristics of a species and a female parasitoid's "perception" of its likely life expectancy.

Ovigeny index Jervis *et al.* (2001) have refined the concepts pro-ovigenic and synovigenic proposed by Flanders (1950) by devising an "ovigeny index." This is defined as the proportion of the maximum potential egg complement that is mature when an adult female emerges into the environment. They designated species that have an index of 1 as "strictly pro-ovigenic" and those species with an index less than 1 as exhibiting varying degrees of "synovigenicity." The ovigenic index has a profound influence on the age-specific fecundity exhibited, especially early in adult life and assuming females have continuous or at least daily access to an abundance of high ranked hosts. Under such conditions, strictly pro-ovigenic species have been found to lay most of their lifetime egg complement within one or two days of emergence (e.g., Fleury and Boulétreau, 1993; Garcia *et al.*, 2001). In contrast, species that have an index of zero (no mature eggs at emergence) have been shown to lay few, if any, eggs one or more days after emergence. After daily egg laying begins, it continues at approximately the same rate over a considerable proportion of the female's life span (e.g., Cohen and Mackauer, 1987; Donaldson and Walter, 1988). Therefore, the proportion of eggs available to be laid early in adult life would be positively correlated with the ovigeny index.

What implications do these differences in reproductive strategies have for the design and interpretation of host range tests? The main one relates to species that emerge with no or very few mature eggs. There is a risk that young females may not oviposit in any host because of a lack of mature eggs. Furthermore, even young females carrying some mature eggs might not oviposit in lower ranked hosts because of a low egg load. This highlights the need in no-choice tests for the use of rigorous positive controls, in which females from the same rearing group are exposed to the target pest to confirm egg laying ability of the parasitoid cohort used.

It is clearly desirable to know approximately the ovigeny index of the parasitoid in question or, at least, the egg load at emergence. In the absence of such knowledge, a possible alternative strategy is to keep female parasitoids for a few days without hosts prior to using them in host range tests. With this strategy, there is a slight risk that strictly pro-ovigenic species might reduce their egg load as a result of resorption (see above). It is relevant to note, in this context, that pro-ovigenic species have a significantly shorter adult life span that synovigenic species (Jervis *et al.*, 2001). Therefore, a standard period of deprivation would constitute a greater proportion of the life span in pro-ovigenic species, increasing their risk of resorption.

Life expectancy At least three studies have provided evidence that female parasitoids with a "perception" of reduced life expectancy display a higher incidence of superparasitism. This implies that such females are more likely to accept parasitized hosts than females of the same age with longer life expectancy. In two studies with *Leptopilina heterotoma* (Thomson), a higher incidence of superparasitism was seen in females that received cues indicative of a shorter life expectancy. The cues provided were a photoperiod typical of autumn, as opposed to a photoperiod typical of mid-summer (Roitberg *et al.*, 1992) and a drop in barometric pressure, an indicator of an imminent storm (Roitberg *et al.*, 1993). In the third study, food-deprived females of *Venturia canescens* (Gravenhorst) were found to have a higher incidence of superparasitism than females that had been fed honey when both groups were tested 24 hours after eclosion (Fletcher *et al.*, 1994). The food-deprived females lived for about 2 days whereas the fed females lived for about 4 days.

The above results suggest that female parasitoids might express a wider host range when their life expectancy is reduced. Thus, it might be beneficial to test females given treatments that reduce their life expectancy (presumably by a period of food deprivation).

EFFECTS OF MATING STATUS

The mating status of a female parasitoid in those species where males commonly exist may also influence responsiveness to host stimuli. For several arrhenotokous aphidiid parasitoids, mated females remained longer in host patches, parasitized more aphids per unit time, and laid more eggs per parasitized host than did virgin females (Michaud and Mackauer, 1995). Parra *et al.* (1996) found that mated females showed enhanced upwind flight in response to the SHC. In those species where both sexes exist and can be easily distinguished, it is highly advisable, therefore, to either ensure mating occurs beforehand or have both sexes present during tests.

EFFECTS OF FEMALE SIZE

It is common in insects that egg load is positively correlated with size for females of similar physiological age and nutritional history. For example, this has been shown in the parasitoids *Aphytis lingnanensis* Compere (Rosenheim and Rosen, 1991) and *Anaphes nitens* Girault (Carbone and Rivera, 2003), and we suspect this would be so in all parasitoid species. As discussed above in the section on effects of host deprivation, it is widely accepted that readiness to oviposit is correlated with egg load (Minkenberg *et al.*, 1992). It is logical to believe, therefore, that there would be a positive relationship between size and readiness to oviposit, even if the variation in readiness to oviposit is mediated by egg load rather than by size *per se* (Rosenheim and Rosen, 1991). This suggests that females used in tests should be as large as possible.

INFLUENCE OF EXPERIENCE AND PHYSIOLOGICAL STATE OF PREDATORS

EFFECTS OF EXPERIENCE

Relatively few studies have investigated the effects of experience on the foraging behavior of predators. Enhanced responsiveness has been demonstrated in the anthocorid *Anthocoris nemoralis* (F.) (Drukker *et al.*, 2000) and the predatory mite *Phytoseiulus persimilis* Athias-Heriot (de Boer and Dicke, 2003) to a previously experienced volatile semiochemical. In addition, it has been shown that host selection in a predacious wasp is influenced by larval/early adult experience (Rayor and Munson, 2002). As these results are strikingly similar to those seen in some parasitoids, we believe that experienced-induced changes in responsiveness could be expected to occur more or less generally in predators. Any bias in favor of the previously consumed species and/or its substrate can be avoided or reduced by analogous strategies that we have suggested for parasitoids (see above).

EFFECTS OF PREY DEPRIVATION

As with phytophagous insects, the likelihood of acceptance of hosts for feeding is expected to increase with increasing periods of food deprivation (Barton Browne and Withers, 2002). The consequence of a deprivation-induced increase in acceptance that is most relevant to host specificity testing is that deprived predators might accept a wider range of hosts than non-deprived individuals would. This will have a direct influence on experimentally deduced host range, and has been demonstrated in stonefly larva preying on mayfly larvae (Molles and Pietruszka, 1983, 1987).

Our present understanding of the influence of food deprivation on host acceptance behavior (Withers *et al.*, 2000; Barton Browne and Withers, 2002) suggests that satiated predators introduced into choice tests would be in danger of showing reduced attack and feeding on less preferred but otherwise acceptable test species. Satiated predators might also potentially fail to accept test species in no-choice tests if the period of access to the non-target species was short. Consequently, such tests would fail to reveal the fundamental host range (sensu Nechols and Kikuchi, 1985; van Klinken, 2000), and there is a risk, therefore, that they would produce a false negative result. Hence a period of food deprivation prior to initiating testing would be strongly advisable.

INFLUENCE OF THE TEST ENVIRONMENT

EFFECTS OF TYPE OF TEST

The attributes of the various kinds of tests are discussed in Chapter 7. Here, we will briefly discuss the instances in which a non-target species is accepted in choice tests including the highly ranked target species and is rejected in no-choice tests (i.e., a wider host range is expressed in choice tests than in no-choice tests) (Marohasy, 1998). As pointed out by Barton Browne and Withers (2002) in their analysis of the effects of time-dependent changes in responsiveness, by Papaj and Rausher (1983), and also in Chapter 7 of this volume, there is a general expectation that a wider host range will be expressed in no-choice tests than in choice tests.

There are, however, at least four known examples in parasitoids in which one or more non-target species have been attacked in choice tests yet not attacked in no-choice tests (Bailey, 1989; Field and Darby, 1991; Barratt *et al.*, 1997; Kitt and Keller, 1998). Two explanations can be suggested to account for this type of occurrence. The first is that volatile kairomones from the highly ranked target species have condensed on or in the immediate vicinity of the non-target species. The second is that stimulation elicited by kairomones of the target species have generated an excitatory state in the female parasitoid's central nervous system, leading her to accept non-target species providing a lower level of stimulation ('central excitation' sensu Dethier *et al.*, 1965).

In one of the examples, the parasitoid *Sphecophaga vesparum* Curtis (Ichneumonidae) oviposited in (and then successfully developed in) two adjacent cells of a non-target wasp when unguarded cells were held in a choice situation within 10 cm of cells of the target wasp, *Vespula* *spp.* In contrast, no-choice tests found no parasitism occurred on the non-target wasp *Ropalidia plebeiana* Richards (Field and Darby, 1991). In the field, these species are unlikely to nest in close proximity, leading to the conclusion that the result of the no-choice test is the true one in this case.

Whether a wider host range is expressed in no-choice or choice tests depends on the relative strengths of any time-dependent effects, on the one hand, and the effects of kairomonal contamination and/or central excitation, on the other. Since there is no way of predicting the outcome of the above processes, it would seem advantageous to perform both no-choice and choice tests including the target species before making conclusions.

EFFECTS OF SIZE AND FORM OF THE TEST ARENA

It is generally accepted that, in the field, there is a sequence of behaviors leading to host location and acceptance. This is especially true of the natural enemies of phytophagous arthropods (Vet *et al.*, 1995). Cues from the host habitat perceived at a distance (e.g., olfactory, visual) are important at the early steps in the sequence, and contact cues (e.g., gustatory) are important later in the sequence, particularly in the final acceptance or rejection stage. In most laboratory testing situations, at least some steps of the early sequence are prevented by the small size and lack of natural complexity of the test arena. There is a possibility, therefore, that the range of hosts accepted in small arenas will be wider in the laboratory than in the field if failure to respond to one or more distance cues is the factor responsible for the non-host status of any species in the field (i.e., a failure to locate the host occurs under natural conditions, which does not occur in the laboratory assay).

The scientist has a choice between attempting to simulate the field situation in host range tests or attempting to provide conditions where the maximal host range is likely to be expressed. We have stated in the introduction that we have recommended taking the latter approach, at least initially, in order to fully assess non-target species at risk of attack (see van Klinken, 2000). We believe it is adequate for the test arena to be small and simple in structure. The main consequence of predicting host range using only the final stages of host location and acceptance is to predict a host range that may be broader than would actually occur in the field (Keller, 1999). Assays incorporating more natural conditions indicative of the field should only need to be employed when such false-positive results (sensu Marohasy, 1998) are strongly suspected.

EFFECT OF PARASITOID DENSITY

It is possible to test parasitoids singly or in groups. The results reported in a recent paper suggests that female parasitoids display a greater readiness to oviposit when in groups than when tested singly. Carbone and Rivera (2003) found that the egg parasitoid *A.* nitens laid 50% more eggs per female when tested in groups than when tested singly. There was also a higher incidence of superparasitism when females were in groups (33% vs. 15%).

This result was interpreted as an adaptive response to the "perception" of competition for hosts. If this is indeed so, the occurrence of this kind of response should be widespread among parasitoid species. In the interests of revealing the widest realistic host range for oviposition, we recommend, therefore, that the parasitoids should be tested in groups rather than singly. Naturally separate experiments using individual females would be required in order to obtain accurate data on attack rates, etc.

EFFECTS OF PROPORTION OF THE TARGET SPECIES RELATIVE TO NON-TARGET SPECIES

The results obtained by Cornell and Pimentel (1978) demonstrate that the outcomes of choice tests may be affected by the relative proportions of test species presented. They found that when the parasitoid *Nasonia vitripennis* (Walker) was given a choice of puparia of two blowfly species, the apparent preference shown for a species was positively correlated with the proportion of that species within the test arena. This phenomenon of frequency-dependent attack rate has implications for the design of choice tests. For example, if the target species were to outnumber one or more test species, there may be a reduced probability of attack on a lower ranked but acceptable non-target species. Thus, the most challenging choice test would one presenting only a small proportion of the presumably high ranked target species compared to the non-target species. In the interests of revealing the widest realistic host range for oviposition, we recommend, therefore, presenting only a small proportion of the presumably high ranked target species compared to the non-target species. The down-side of this approach is that statistical analysis will be compromised compared to situations in which equal proportions of test species are presented.

CONCLUSIONS AND RECOMMENDATIONS

Given the overall objective to maximize the probability of attack on non-target species in a laboratory test, we recommend the following practices or conditions.

FOR TESTS WITH PARASITOIDS

1. To take account of the potentially opposing effects of various behavioral and physiological processes:
    - Perform no-choice tests with both naive and oviposition-experienced females because it has been shown that oviposition experience can reduce responsiveness (through a specific learning process) or enhance responsiveness (through priming).
    - Perform both choice and no-choice tests because parasitoids can display wider host ranges in choice tests (contrary to the general expectation) because of time-dependent processes.
2. To minimize any experienced-induced bias in favor of the rearing host, particularly in the context of choice tests:
    - Rear parasitoids on a host other than the target species, whenever possible.
3. To minimize any experience-induced bias in the favor of the rearing host's substrate (food), particularly in the context of choice tests:
    - Rear parasitoids on hosts on a different substrate from those used in the test.
    - Present target and non-target species on an inert surface whenever possible.

4. To avoid any experience-induced bias in favor of rearing SHC or components thereof due to continuing contact with the rearing environment after eclosion, particularly in the context of choice tests:
   - Remove parasitoid from the rearing environment before or shortly after eclosion.

5. To take advantage of any increase in readiness to oviposit induced by host deprivation *per se* and/or any associated changes in egg load:
   - Keep parasitoids separate from hosts but with a source of suitable food for a few days after eclosion before the test, especially in synovigenic species with a low ovigeny index.
   - If oviposition is permitted, allow a period of host deprivation before the test.
   - In host feeding species, provide a source of nitrogen-containing food (in addition to carbohydrate) during a period of host deprivation.

6. To take advantage of any increase in readiness to oviposit induced by a perception of competition for hosts:
   - Test parasitoids in groups rather than singly.

7. To take advantage of any increase in readiness to oviposit induced by a perception of a reduction in life-expectancy:
   - Subject females to a period of food deprivation before using in tests.

8. To take advantage of any increased expressed host range in environments that do not require parasitoids to respond to distance cues to establish contact with potential hosts:
   - Use small arenas that are simple in structure.

9. To take advantage of the relationship between female size and egg load and of any correlation between egg load and readiness to oviposit:
   - Use females that are as large as possible.

10. To take advantage of any frequency-dependent attack rate:
    - Provide only a small proportion of the target species compared to non-target species in choice tests.

FOR TESTS WITH PREDATORS

1. To take advantage of any increase in the tendency for food-deprived insects to accept lower ranked food:
   - Deprive predators of prey for a period before the test.

2. To minimize any experience-induced bias in favor the target species, especially in the context of choice tests:
   - Rear and maintain predators on a species other than the target species, if possible.

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CHAPTER 6. PARAMETERS USED IN LABORATORY HOST RANGE TESTS

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INTRODUCTION

Here, we discuss specific parameters that can be used to characterized the responses – oviposition, feeding, survival, and development – of parasitoids and predators in tests to estimate their host ranges. For any such tests to give reproducible results, both the physical setup, the prey, and the predator much be held to a defined set of conditions. Such factors and how they can affect results of laboratory host range tests are considered in Chapter 5. In Chapter 7, we discuss the various test designs that have been used in host range estimation.

IF YOU ARE WORKING WITH PREDATORS

Unlike parasitoids, for predators, both adults and larvae are mobile and can actively seek prey. Thus, each stage's host range must be assessed, as they may differ. When working with predators, four processes can be observed: (1) feeding (by adults or larvae), (2) adult survival, (3) oviposition (including oogenesis), and (4) larval development. We also discuss likely effects of predator fidelity to habitat type or host plant species on field prey range.

FEEDING (ADULTS AND LARVAE)

Using standard conditions, the quality of a prey species for the predator can be quantified by measuring the number of prey eaten per predator per unit of time (Parameter 1). For both adults and larvae, prior experience with a prey may condition the response in a test. Consequently, both naïve insects and ones conditioned to the target pest should be examined as separate treatments (see Chapter 5)

Parameter 1: Number of prey eaten per predator per unit of time

For easily counted prey, the most direct measure of prey acceptance by the predator is to count the number eaten in a laboratory assay in a standard amount of time (usually 24 hours or some lesser period) and compare this to the number of the target pest, or other test species, consumed. For example, Miller and Williams (1983) compared the number of eggs of each of nine prey species eaten by the staphylinid beetle *Atheta coriaria* (Kraatz) in choice tests where the predator was offered one egg of each of three host species for 24 hours. Similarly, Zilahi-Balogh *et al.* (2002) compared the number of eggs eaten by the derodontid beetle *Laricobius nigrinus* Fender when adults were presented with eggs of either the target pest (*Adelges tsugae* Annand), other adelgids, or scales. They found that the numbers of nontarget prey eggs eaten in a three-day no-choice test were only 14 to 51% of the number of target pest eggs eaten under the same conditions. In such tests, it is important to include negative controls (arenas with no predators) to estimate numbers of prey that die or disappear from causes other than predation and positive controls (arenas in which the predator is presented with the target pest) to demonstrate the predator was physiologically ready to consume prey.

Prey consumed by predatory larvae can be measured using methods similar to those discussed above. Prey choices, however, may differ between young and old larvae; therefore, larvae of different ages should be tested as separate treatments (see Chapter 5). Young larvae, for example, may require a more particular prey species or even prey life stage, while older larvae may feed on a wider range of prey. In chewing species, for example, this may be due in part to lower biting strength of young larvae.

ADULT SURVIVAL

If prey are not easily counted, it may be more effective to measure survival times of predators fed pure diets of single test species (Parameter 2). This approach is useful, for example, (1) for prey such as scales that occur in congested colonies, (2) when prey molt to the next life stage during the test (e.g., Causton *et al.*, 2004), or (3) when uncountable reproduction occurs during the test period (such as with some adelgids), changing the number of prey presented (Butin, 2003).

Parameter 2: Predator survival (in days) when fed only a given prey

For adult predators, another measure of the value of a potential prey is the average number of days a newly emerged, naïve adult predator lives when confined with that test species and water, compared to the survival when confined with (1) the target pest and water or (2) water only. For larvae, this test is approximated by measuring survival to the next life stage (as discussed below in parameter 4a). A test species should be considered a prey only if eating it raises the predator's survival to values higher than on water alone. A test species would be considered a prey of lesser value if predator survival on the test species was greater than on water alone, but less than that on the target pest. Lopez and Kairo (2003), for example, found that survival times for both adults and larvae of the coccinellid *Nephaspis bicolor* Gordon fed only non-whitefly prey were no better than those of starved controls held on moist filter paper. These data suggest that the prey range of this coccinellid is limited to whitefly species.

OVIPOSITION

The value of a test species for predator ovipostion can be assessed by answering three questions. (1) Can the predator develop mature eggs when fed only the test species (Parameter 3a)? (2) Does the test species stimulate the predator to lay its eggs (Parameter 3b)? (3) How many eggs does the predator lay when provided access only to the test species compared to when provided access only to the target pest (Parameter 3c)? Since predators may produce fewer eggs when very young or old, predator age should be considered in test design.

Parameter 3a. Egg development (oogenisis) by adults

The nutritional value of a prey species can be measured by its ability to support the development of mature eggs when it serves as the sole food of the adult predator. This can be determined by holding two groups of newly emerged adults (reared as larvae on the target pest) under the physical conditions and length of time that would lead to oviposition on the target pest, giving one group access to only the target pest and confining the other group with a different prey species. Periodically, a subsample of the predators can be dissected and egg development compared between the two groups. The group with access to the target pest serves as the positive control. A third group, held only with water and non-prey foods such as honey, serves as the negative control.

Parameter 3b. Ability of prey to elicit predator oviposition

Many predators lay eggs when they contact stimuli from particular prey. Lopez and Kairo (2003), for example, found that the coccinellid *N. bicolor* lays its eggs in response to wax from its whitefly prey. If a predator only oviposits in response to such a stimulus, its larvae will have access only to prey with those characteristics; larvae of *N. bicolor*, for example, would therefore be expected to be found eating whiteflies in nature. When key kairomones are lacking, oviposition (on a novel prey) is likely to be absent or much reduced. Albuquerque *et al.* (1997) found that oviposition by the specialist green lacewing *Chrysopa slossonae* Banks on novel prey (aphids other than the woolly alder aphid, *Prociphilus tesselatus* [Fitch]) was one-third of that on its usual prey, woolly alder aphid. The key to effective use of this test is recognizing some substance associated with a prey species that is a specific releaser of oviposition. Proof of its nature can be had if transfer of that substance to a related species not normally used by naïve predators for oviposition induces them to lay eggs on the amended nonhost species.

DEVELOPMENT OF IMMATURE STAGES

Host ranges of larvae sometimes differ from those of adults of the same predator species and should be determined separately. The prey range of predator larvae can be measured in terms of larval survival and development (Parameter 4a) and size and fecundity of the adults obtained (Parameter 4b) when reared as larvae on a test species.

Parameter 3c. Numbers of eggs laid

Finally, the number of eggs laid in response to the presence of a test species in a no-choice design provides further information on the likelihood that the species would be used as a prey. To measure this effect, predators with developed eggs should be placed in a standard test arena with a prey species and the number of eggs laid in a fixed period counted and compared to the number laid in the presence of the target pest under the same conditions. Since prior exposure to the pest species is a confounding effect, such conditioned predators should be used only if this is the only means to obtain predators with mature eggs; otherwise, naïve predators should be used. A control treatment (no prey of any species) should also be used to account for the potential for egg dumping in the absence of prey-related cues (e.g., Causton *et al.*, 2004). Zilahi-Balogh *et al.* (2002) found that, in no-choice tests with various nontarget species, field-collected derodontid beetles (*L. nigrinus*) laid on average only 16% of the number of eggs laid when exposed to the target pest (*A. tsugae*) under the same conditions; in paired choice tests, this dropped further to only 6%. Since field collected beetles had previously fed on the target pest, the results of both tests are confounded by preconditioning to the preferred prey.

Parameter 4a. Larval survival and rate of development

The quality of a prey species to a larval predator can be assessed by measuring the percentage of a larval cohort that survive to pupation (or for hemimetabolous insects, molt to the adult) on a diet of the test species versus on the target pest (the positive control) or on water alone. Zilahi-Balogh *et al.* (2002) found that 14% of eggs of the derodontid beetle *L. nigrinus* survived to the adult stage when larvae were fed on hemlock woolly adelgid, but this dropped to zero for all the other five species of prey tested. The time needed for 100% development of the immature stage (at a standard constant temperature) can also be used as an index of prey suitability, as slower development is expected on prey of lower quality.

Parameter 4b. Weight and fecundity of adults reared on test species

As better foods should lead to heavier body weights – and therefore greater fecundity – larval, pupal and adult weights of predators fed as larvae on various test prey can be compared to that of predators reared on the target pest species as an estimate of prey quality. As it may be difficult to weigh very small predators without killing them, this assessment may need to be done by weighing groups of a fixed number of predators taken from batches reared on different larval diets.

Effects of larval diet on adult fecundity can be measured by offering adults reared as larvae either on the target pest or on a non-target test species batches of the target species for oviposition and comparing the number of eggs laid between the groups with different larval diet histories. Tests should be kept short (24 and 48 hours) to avoid influences due to any consumption of the target pest during the test.

EFFECTS OF PREDATOR FIDELITY TO HABITAT OR PLANT SPECIES ON FIELD PREY RANGE

As with parasitoids, if a predator exhibits high fidelity to particular habitats or plants, then these features can act as filters narrowing the predator's field prey range. This is the case, for example, with some species of phytoseiid mites. Beard and Walter (2001) found that species of *Neoseiulus* in inland Australia, while considered to be generalist predators, in fact showed high fidelity to particular tree species or small groups of species. Of the 73 examples of *Neoseiulus eremitus* Beard that were collected, all were from only one tree species (*Eremophila mitchelli* Benth.), and all of the 149 specimens of *Neoseiulus buxeus* Beard were collected from *Eucalyptus populnea* F. Muell. Fidelity at the habitat level has been shown by Walter *et al.* (1998), who deployed spider mite prey colonies in tropical Australian habitats to map the presence of the introduced predator mite *Phytoseiulus persimilis* Athias-Henriot and found that this predator, while established in the wild in Australia, did not enter forested habitats.

Laboratory tests are not easily used to observe these processes, especially habitat fidelity. Olfactometers might be useful in establishing the level of responsiveness of particular predators to particular plants. Even a simple choice test in which two plant species are presented together in a small arena and the predator's later position noted can suggest potential predator ties to particular plant species or groups. However, in a still air assay, mixing of volatiles from several test plants may occur and blur the difference between the treatments. Demonstration of plant fidelity is likely to require larger scale tests, with moving air.

Investigation into habitat or host plant selectivity would be needed, especially in cases in which field surveys recorded the predator only in specific habitats or on certain host plants but feeding or oviposition was observed in the laboratory on prey from other habitats or plants.

IF YOU ARE WORKING WITH PARASITOIDS

For most parasitoid species, hosts are found by adult females. The adult's host searching process, therefore, determines which host species are attacked. A great deal has been learned since the 1960s about the mechanisms by which female parasitoids locate and choose hosts (see Godfray, 1994; Jervis and Kidd, 1996; Quicke, 1997). This process can be broken into several steps – host finding, host acceptance, and regulation of host physiology – each of which offers opportunity for measurements useful in assessing a species' host range.

HOST FINDING

Detection of a suitable host can be divided into a series of stages, the first being habitat location; the second, finding of the insect's particular host plant; and the last, discovery of the insect itself on the host plant. At each step, physiologically suitable hosts may be omitted from the host range if a particular species' habitat is not searched, its host plant is not located, or the host is not found when the agent is foraging on the host plant.

Finding the habitat Parasitoid habitat preferences can determine which species are encountered by a parasitoid. For example, the braconid *Cotesia glomerata* (L.), a species introduced to North America from Europe, does not attack the native nontarget woodland butterfly *Pieris* *virginiensis* Edwards in New England because this parasitoid does not enter woods to search for hosts (Benson *et al.*, 2003a). In contrast, another native woodland species, *Pieris napi oleracea* Harris, has its second brood in meadows and is attacked by *C. glomerata* (Benson *et al.*, 2003b).

There is, however, no obvious way to determine a parasitoid's habitat preferences in the laboratory before introduction. A partial determination can be made by pre-introduction studies of habitat associations in the parasitoid's native range, as in the case of studies of European mirid bug parasitoids being conducted in support of their possible introduction into North America (Kuhlman *et al.*, 2000). In field surveys, however, the effects of habitat itself may be confounded by plant and host insect effects. If, for example, a certain habitat in the native range lacks suitable plants to support hosts, then surveys in that habitat will likely not detect any parasitoids. However, if suitable plants are present in the same habitat in the receiving country, then the parasitoid may enter that habitat. In some cases, if there is a known volatile attractant from the plant/host complex of the typical host of a parasitoid, that compound can be used to bait traps to survey habitats to detect a target parasitoid. *Cotesia glomerata*, for example, can be detected with yellow sticky cards baited with beta-glucosidae (Mattiacci *et al.*, 1995).

One should not assume that, just because the target pest is found in an agricultural habitat, a parasitoid used against it will also be limited to such agricultural areas. The braconid *Microctonus aethiopoides* Loan, for example, after its introduction to New Zealand for control of pest weevils in alfalfa fields, was found in a variety of habitats, including modified native grasslands in subalpine zones, where it parasitized several native weevils (Barratt *et al.*, 1998) (see also Chapter 9).

Responding to the insect/plant volatiles A large body of research over the last 40 years has elaborately demonstrated that plant chemistry and morphology affect host finding by natural enemies, especially parasitoids (Cortesero *et al.*, 2000). Parasitoids' abilities to orient towards hosts from a distance are often based on detection of volatile compounds produced by plants, often in response to herbivore feeding (e.g., Read *et al.*, 1970; Navasero and Elzen, 1989; Roland *et al.*, 1989; Turlings *et al.*, 1991; Wickremasinghe and van Emden, 1992; Romeis *et al.*, 1997; Rutledge and Wiedenmann, 1999). Consequently, the plant that the herbivore feeds on can mediate the insect's risk of discovery and parasitism. The same herbivore on different plants can trigger the release of different volatile blends, as can different herbivores on the same plant. This process means that some physiologically acceptable hosts will escape parasitism simply because the right volatiles are not present for the parasitoid to detect. For example, colonies of green peach aphid (*Myzus persicae* [Sulzer]) feeding on collards (*Brassicae oleracea* L.) were parasitized by the braconid *Diaeretiella rapae* (McIntosh) at a markedly higher rate than was the same aphid species on sugar beet (*Beta vulgaris* L.) because of this parasitoid's attraction to the essential constituent of mustard oil (allyl isothiocyanate), which is present in collards but not beets (Read *et al.*, 1970).

Similarly, variation between plant species in such physical features as leaf trichome density can mean that parasitism is absent or much less frequent in hosts on plants with unfavorable features, even if the insects are detectable by foraging parasitoids (e.g., Turner, 1983; Hua *et al.*, 1987).

Finally, variation in secondary compounds can render herbivores on some plant species unacceptable for oviposition (Sime, 2002) or unsuitable for development of immature parasitoids (Kester and Barbosa, 1991) due to the sequestration of toxic plant compounds by the insect larvae as they feed. For example, Sime (2002) found that the ichneumonid *Trogus pennator* (Fabricius) did not parasitize the Troidini swallowtail butterfly *Battus philenor* (L.) even though its frass did attract the parasitoid. Rejection of larvae was attributed to the presence of ethanol-soluble compounds (in part, at least, aristolochic acids sequestered from the host plant), which were found on the external surface of the larval integument. Furthermore, in those few cases in which the parasitoids could be induced to oviposit in *B. philenor* larvae, parasitoid progeny died. This case illustrates the likely role of plant chemistry in shaping the parasitoid associations of swallowtails, since *Trogus* spp. readily attack species in two of the three papilionid tribes, but not those in the Troidini, whose members are distinguished by their use of plants in the Aristolochiaceae, which contain aristolochic acids. (The same phenomenon occurs with predators: the Vedalia beetle [*R. cardinalis*] does not feed on prey that have fed on plants with certain alkaloids [Quezada, 1969; Mendel *et al.*, 1992]).

The consequence of these plant effects is twofold. On one hand, some native herbivores that are in a parasitoid's physiological host range will not be used as hosts in the field if they occur on unattractive or morphologically unsuitable plants. Conversely, native herbivores that expand their own host ranges by moving onto introduced plants may become new field hosts of additional parasitoids that search those plants. Babendreier *et al.* (2003a) found that some combinations of influences of host plant species and habitat complexity lowered parasitism by *Trichogramma brassicae* Bezdenko on sentinel eggs of the rearing host (*Ephestia kuehniella* Zeller) in meadows, compared to corn fields.

In laboratory tests designed to predict which native insects might become field hosts for a candidate natural enemy, it is important to (1) test native herbivores on their typical host plants and (2) use test arenas large enough that long-distance host finding is a required step in parasitism. Wind tunnels provide enough space for active upwind flight of parasitoids and are a good arena for assessing the above points (Parameter 5).

Parameter 5: Successful upwind flight to a herbivore/plant complex

The ability of a native herbivore on a native plant to attract upwind parasitoid flight leading to host discovery can be scored as the percentage of female parasitoids that succeed in reaching a bait (consisting of the correct herbivore stage on its native host plant) in a wind tunnel, together with the time taken to reach the bait (Keller, 1990, 1999; Geervliet *et al.*, 1996). Species that do not elicit oriented upwind flight and high discovery rates are unlikely to be exploited in the field. Using this test, species that are physiologically suitable as hosts but are not associated with sufficiently attractive volatiles can be recognized as nonhosts. For species feeding on several plants, parasitism may be high on some species (ones producing attractive volatiles) and low or absent on others (not producing attractive volatiles) (Read *et al.*, 1970; Roland *et al.*, 1989). Oviposition by the tachinid *Cyzenis albicans* (Fall.) was low on apple trees with winter moth (*Operophtera brumata* [L.]) because attractive volatiles produced by oaks were not produced by apple. Spraying of apple trees with oak leaf extracts in small field plots doubled the number of parasitoid eggs laid on the treated apple trees compared to untreated controls (Roland *et al.*, 1989).

Host kairomone effects Once a parasitoid has found a plant with a potential host insect on it, the parasitoid engages in intensified local search to reach the actual insect. Responsiveness to chemicals (Parameter 6) found in such materials as insect body parts (scales, setae, cast skins), excretions (honeydew, silk), and herbivore-damaged plant tissue helps the parasitoid locate the host. Contact with these chemicals (kairomones) induces parasitoid behaviors such as more frequent turning, slower walking, and lower rates of departure by flight, which have the effect of keeping the parasitoid searching the local area.

Parameter 6: Parasitoid responsiveness to a test species' kairomones

Parasitism of a test species in nature at high levels is unlikely unless the parasitoid is responsive to the species' contact kairomones (any source of non-volatile chemical cues perceived by physical contact), which often are what guide the parasitoid to the host's exact location after the parasitoid lands on an infested plant. Potency of a species' kairomones can be assessed in the laboratory by determining the degree to which they arrest parasitoid movement and/or induce oviposition, compared to those of the target pest.

HOST ACCEPTANCE

After physical contact is made with a host, the parasitoid continues to gain further information by examining the potential host with her antennae. If the parasitoid is sufficiently stimulated, she will attempt to oviposit in or on the host (Parameter 7). For example, the aphelinid *Aphytis melinus* DeBach, a parasitoid of California red scale, *Aonidiella aurantii* (Maskell), recognizes its host by detecting the chemical *O*-caffeoyltyrosine on the scale cover. Parasitoids acquire sensitivity to this compound by contacting it when adults emerge from their natal hosts. Subsequent contact with this chemical on scales triggers host acceptance in ovipositing females (Hare, 1996). During ovipositor insertion, further information about the host is gained by parasitoids through sensilla on the ovipositor, and hosts may be rejected even at this stage.

Parameter 7: Parasitism rate

If test results have shown that (1) a parasitoid is able to detect odors from a test species on its natural host plant and fly upwind or in some other way orient to it from a distance and (2) that the parasitoid is responsive to the species' kairomones, then parasitism is a meaningful parameter to measure as an assessment of host range. Rates of parasitism in various kinds of tests (choice, no-choice, sequential, see Chapter 7) can be measured and compared to that on the target pest. For example, Babendreier *et al.* (2003b) assessed rates of parasitism by *T. brassicae* in a variety of nontarget species using no-choice, small arena, dead air tests.

REGULATION OF THE HOST'S PHYSIOLOGY

Once a parasitoid has found and parasitized a host, it must defeat all attempts of the host to destroy it and also regulate the host's physiology in ways that render it favorable for the survival and growth of the immature parasitoids. Host defenses such as encapsulation (Parameter 8) provide several more points at which measurements can be made that describe the quality of a species as a host for a particular parasitoid. In general it is assumed that, if a host species is not suitable for the survival and growth of the immature parasitoid, the species is not threatened because it will not support a population of the parasitoid. Keller (1999), for example, found parasitism was unsuccessful in many of the test species in which the parasitoids deposited eggs.

Parameter 8: Rate of encapsulation by the host

Encapsulation is a common reaction in which hosts attempt to kill eggs or larvae of internal parasitoids by entombing them inside a layer of material formed from blood cells (Nappi, 1973). This layer of collapsed blood cells often turns dark, and thus can easily be observed. Rates of encapsulation determine if a particular host is suitable or not for a given parasitoid. Blumberg and Van Driesche (2001), for example, found that the obscure mealybug (*Pseudococcus viburni* [Signoret]) was able to encapsulate all of the eggs of *Leptomastix dactylopii* Howard, making this a nonhost for the parasitoid, in contrast to the complete absence of encapsulation in the normal host, citrus mealybug (*Planococcus citri* Risso). Encapsulation rates are readily measured in the laboratory by dissecting test species after exposure to parasitoids. Rates of encapsulation, however, are affected by the exact host life stage (instar) and rearing temperature, in addition to the host species, and these factors must be either considered or held constant. Furthermore, wasps in the families Braconidae and Ichneumonidae have viral symbionts (Polydnaviridae species) that can suppress host encapsulation responses (Edson *et al.*, 1981; Beckage, 1998), and thus are a further influence in determining the usual host range.

One might think that it would be possible to predict the rate of host encapsulation in a particular host based on rates seen in that host with other parasitoids. Similarly, it might seem feasible to predict encapsulation probability for a given parasitoid based on data from other hosts. Neither of these propositions, however, turn out to be true. Closely related hosts can differ widely in their response to the same parasitoid, and a single host can respond quite differently to closely related parasitoids (see Alleyne and Wiedenmann, [2001] for a case study). Thus encapsulation rates are useful measures of host suitability but are not predictable and require the testing of each host-parasitoid combination of interest.

Conversely, not all hosts in which the parasitoid develops successfully in the laboratory are actual field hosts. This is especially true for idiobiont hymenopteran parasitoids and some dipterans (phorids and some tachinids), which interact less intimately with their hosts' physiology than do larval koinobiont parasitoids, which must survive the host's internal defenses. For example, Morehead and Feener (2000) found that the phorid *Apocephalus paraponerae* Borgmeier, which in the field is monophagous on the ant *Paraponera clavata* Fabricius, can develop in at least seven other species in the Ponerinae if eggs are artificially placed in hosts.

Finally, the rate of parasitoid emergence from a test host and the size of the emerging parasitoids (Parameter 9), as well as the sex ratio and fecundity of the emerging parasitoids (Parameter 10) are means to assess the quality of the test species as a host.

Parameter 9: Emergence and size of parasitoid progeny

Hosts that are successfully parasitized and that lack high rates of encapsulation, may still vary in their nutritional value for parasitoid immature stages. Rates of survival of these stages can be measured in laboratory rearings. Three values can be assessed: (1) the proportion of oviposition attempts that result in actual oviposition, (2) the number of progeny per host (for gregarious parasitoids), and (3) the size of the progeny (often using hind femur length as a correlate of body size). If a parasitoid fails to develop on a species in such a laboratory test, the species is likely not a host. The meaning, however, of differing levels of survival is harder to interpret.

Parameter 10: Sex ratio and fecundity of parasitoid progeny

Sex ratio and fecundity of parasitoid progeny reared from a given test host can be used as a further measure of the quality of that species as a host. Male-biased sex ratios suggest low host quality, as does reduced female size compared to size of females reared from the target pest. Smaller females, or ones with shorter lifespans, will produce fewer progeny of their own.

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CHAPTER 7. OVERVIEW OF TESTING SCHEMES AND DESIGNS USED TO ESTIMATE HOST RANGES

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INTRODUCTION

Host range estimation for parasitoids and predacious insects draws on two bodies of past work: work with herbivorous insects used as weed biological control agents and basic studies of how entomophagous insects find, assess, and use hosts. Much of the following discussion on the relative merits of different types of tests comes from the weed biological control literature, in which there has been a lively debate about test methods for several decades – in contrast to the relative paucity of such debate for tests with entomophagous insects.

Some authors make a distinction between "host range" and "host specificity," in which they use the former to mean the full list of host species attacked by an agent and host specificity to mean the relative degree of use likely for each of these hosts. Here, we focus on predicting only whether or not a test species is a possible field host (i.e., in the host range). Predicting the relative degree of use that is likely in the field is a more complex task, which weed biological control practitioners have approached by use of preference and, to a lesser degree, continuation tests. An herbivore may, for example, feed on six plants species, but show a strong preference for one species if given the choice. A critical question, however, is "will choice always be available?" We assert that it will not, and therefore even low rank hosts are potentially at risk. However, if continuation tests show that a low ranked host is not suitable for permanent maintenance of the biological control agent's population across many generations, then it is legitimate to argue that such low ranked hosts are indeed not threatened and may be considered non-hosts.

Use of tests to assess plants as potential hosts for herbivorous insects began over 70 years ago and has long been routine. In contrast, interest in estimating parasitoid and predator host ranges lagged considerably behind. For herbivorous weed control agents, a variety of tests have been developed, and those in favor have changed over time due to changes in the perspective of biological control practitioners and developments in the study of insect behavior. For herbivorous insects, tests used have focused on (1) oviposition, (2) adult feeding, (3) larval feeding and survival, (4) oogenesis and multi-generation population persistence, and (5) host preference (see Chapter 6 for a discussion of these parameters). These processes have been examined using tests with several different designs, principally (1) no-choice tests, (2) choice tests, and (3) open field tests. Individual projects often have used several types of tests in various combinations, including various kinds of controls. Tests used less often have included continuation tests, sequential choice tests (sometimes called sequential no-choice tests, see Chapter 13 [Combs]), preference-ranking tests (including a variation called "choice minus target"), and tests that investigate effects of specific aspects of insect behavior (time-dependent effects and behavior-dependent effects). In the following discussion, each of the major types of tests is discussed separately.

NO-CHOICE TESTS

DESIGN

These tests combine one or more specimens of the biological control agent with a single test species, for a fixed period of time, in cages (including petri dishes, plastic containers, or cages of various sizes) under standard laboratory conditions. Thus, if five non-target species are to be tested at one time with ten replicates of each, 50 cages would be required, plus appropriate controls.

As used in the past, test insects in no-choice design experiments may or may not have had contact with the target pest before the test. However, prior experience is a confounding effect because it may reduce response to lower ranked hosts, even if the preferred host is not present during the actual test. Thus, it may be better to avoid this complication and work with naive insects that have not touched or fed on the target pest (or for parasitoids have not had any host contacts or oviposition experiences) (see Chapter 5 for a discussion of such confounding factors).

Interestingly, scientists studying weed biological control agents and those studying parasitoids have treated this issue of prior experience differently. Most weed biological control practitioners assume that prior experience with the target weed will almost always automatically occur in the course of the insect's life cycle, and thus they treat it as a given rather than examine its effects as a treatment variable in tests. Biologists studying parasitoids, in contrast, have shown extensive concern about the effects of prior experience and have routinely treated it as an experimental factor to be controlled and contrasted in experiments on parasitoids' host preferences. This difference in assumptions affects how choice vs. no-choice tests are viewed by these two groups.

For oviposition and feeding trials, positive controls are essential to validate negative responses by showing that the group from which the test biological control agents were taken had the capacity for oviposition or feeding. Individuals used in controls should either be (1) different insects from the same rearing batch that are exposed to the target pest simultaneously with the main experiment or (2) the same insects used in the main experiment that, after exposure to a nontarget species, are re-used by exposing them to the target host to demonstrate their physiological readiness to respond positively to a highly ranked host. Negative controls, in which test species are not exposed to parasitoids, are needed to detect mortality of test herbivores that is unrelated to the parasitoids (see Chapter 8 [Froud and Stevens] for an example). For feeding tests with predators, controls in which only water is provided show whether the test prey species provides any nutritional benefit to the predator by assessing survival time with prey versus water alone (Causton *et al.*, 2004; and Causton, this volume). For tests measuring survival and development of immature parasitoids or predators, performance on a host (or prey) of known suitability can be used as a standard against which reduced survival in a poorer host can be gauged.

HISTORY OF USE

Weed biocontrol agents The no-choice test design was the first approach used in early weed biological control projects and was the dominant method employed until the 1960s (e.g., see Briese, 1989; Thompson and Habeck, 1989; Turner *et al.*, 1990; Adair and Scott, 1993, 1997; Woodburn, 1993; Turner, 1994; Balciunas *et al.*, 1996; Peschken *et al.*, 1997; Scott and Yeoh, 1998). No-choice tests can be used with adults to assess feeding and oviposition and with larvae to assess feeding and development. In all tests, care must be taken to offer the appropriate plant stage to the test insects. For species with larvae that do not move between plants, use of no-choice tests is really the only appropriate model of the field biology since no host choice is exercised by the larvae. In early days, most weed biological control projects included larval feeding tests with no-choice designs.

It was observed, however, that in this type of test, immature herbivorous insects sometimes fed successfully on plant species that adult insects did not find or accept for oviposition. This observation caused emphasis in the testing of weed control agents to shift to the use of tests that focused on the oviposition choices of adults, as this step was believed to be the one that most often limited the host range. This shift toward oviposition tests was intended to reduce the chances that beneficial and safe species would be rejected for introduction because they were seen as unsafe based solely on data from no-choice larval feeding tests.

It was also observed that the range of plants insects laid eggs on was often smaller if a preferred plant (usually the target weed) was present in the test, as opposed to no-choice tests in which plants were each presented alone (e.g., Fornasari *et al.*, 1991; Willson and Garcia, 1992). This led to a strong preference on the part of biological weed control researchers to use choice designs in oviposition tests, starting around the 1970s. In some projects, preference for the target species in such choice oviposition tests was used to argue that attacks on less preferred nontarget species would be minimal in the field.

More recently, it has been recognized that such choice oviposition tests may not detect less favored hosts. For example, a seed bruchid that was recently introduced into New Zealand and Australia for control of broom (*Cytisus scoparius* [L.]) has been found attacking the non-target plant tagasaste (*Chamaecytisus palmensis* [L. Fil.] Link). While this plant was in the list of species tested to estimate the host range of this beetle, its status as a host was not detected (Fowler *et al.*, 2000). This occurred because only choice tests were used; when paired with the target weed, tagasaste was not attacked. A no-choice test would, presumably, have detected this species' status as a possible host. To prevent such errors, no-choice tests are being re-emphasized in weed biological control projects (Heard, 2000), especially by conservation groups and regulatory agencies (Hill, 1999). Such use of no-choice tests also avoids the mixing of plant volatiles, which may mask the identity of co-presented plant species. This is likely also to be relevant to tests with entomophagous insects since their prey or hosts are often presented to them on host plants.

Entomophagous species Here we consider tests for (a) larval development, (b) oviposition, and (c) feeding by adult predators.

(A) Larval development tests For parasitoids, assessment of larval development (the analog to larval starvation tests with herbivores) is typically done by observing whether parasitoids develop and emerge from a test species that had been accepted by adult parasitoids in an oviposition test (e.g., Field and Darby, 1991; López-Vaamonde and Moore, 1998). Occasionally, immature stages of the parasitoid are artificially placed in or on the test host, as Fuester *et al.* [2001] did with eggs of the tachinid *Aphantorhaphopsis samarensis* [Villeneuve]) and Morehead and Feener (2000) did with eggs of the phorid ant parasitoid *Apocephalus paraponerae* Borgmeier, but this is the exception.

For predators (and a few parasitoids, such as those tachinids that scatter microtype eggs on foliage), larval development tests do not depend on successful oviposition on the test species because predator larvae are generally mobile and can readily be placed together with a candidate prey species to see if feeding occurs (Causton *et al.*, 2004).

Unlike the results seen with tests of herbivorous insects in which the host range found in larval feeding is often broader than the range of species accepted by adults for oviposition, the opposite is sometimes the case for parasitoids. For example, Bailey (1989) found the sciomyzid fly *Pelidnoptera nirgripennis* (F.) laid eggs on the integument of millipede species in five families, but larval penetration only occurred in one family (Julidiae), in part because eggs on some species failed to firmly adhere. Duan and Messing (2000) found that the restrictive step determining the host range of the braconid wasp *Dichasmimorpha kraussii* (Fullaway) was failure of immature stages to survive in plant-galling (as opposed to fruit-infesting) tephritids rather than rejection of the galling tephritids by ovipositing adults. Keller (1999) found that the aphid parasitoid *Aphidius rosae* Haliday, under choice conditions, attacked all four species of aphids tested but failed to develop in three of them. Mohamed *et al.* (2003) found that the braconid *Psyttalia cosyrae* (Wilkinson) parasitized all six fruit flies presented, but due to encapsulation, the parasitoid developed in only two species. Similarly, the predatory derodontid beetle *Laricobius nigrinus* Fender oviposited on five species on which larvae failed to develop (Zilahi-Balogh *et al.*, 2002).

In conclusion, while plant tissues may be broadly eatable for herbivores (assuming no strong deterrent compounds or morphological barriers), the internal environment of host insects for parasitoids may require special adaptation for successful exploitation. Thus, no-choice larval development tests may be of greater value for predicting parasitoids' host ranges than larval starvation tests are for herbivorous insects. For idiobiont parasitoids (egg parasitoids and larval/pupal ectoparasitoids) and predators, this would not be the case, as there would be no requirement to defeat internal physiological host defenses.

(B) Oviposition tests Oviposition tests can be applied to both parasitoids and predators, and while these have many common features, there are important differences: chiefly, that predators oviposit near prey or in the prey habitat rather than in or on the host, as parasitoids usually do. There are two key elements in oviposition tests: host finding and host acceptance. Oviposition tests have frequently dealt only with host acceptance because they have been run in small cages that make expression of the full range of host finding behaviors unnecessary. Test cages can be as small as petri dishes (as used to assess the host range of *Trichogramma brassicae* Bezdenko by Babendreier *et al.* [2003]) or, more typically, are small plastic boxes or cages (Bailey, 1989; Field and Darby, 1991; López-Vaamonde and Moore, 1998; Porter and Alonso, 1999; Porter, 2000; Zilahi-Balogh *et al.*, 2002; Van Driesche *et al.*, 2003). Choice of the arena used will depend on the biology of the insect studied and the conditions needed to ensure natural behavior by the agent (see Chapter 5).

Aspects of host finding can be introduced into the testing process by including more space in the test arena, food for adult parasitoids (or predators), appropriate host plants of the test species, and circulating air. Duan and Messing (2000), for example, conducted their tests with tephritid parasitoids in larger cages (1 m on a side) in which hosts and host plants were placed. Keller (1999) conducted his assessment of the host range of a rose aphid parasitoid in a wind tunnel, which provided moving air and sufficient physical space so that flight toward the test aphid/plant complex was a required part of any positive responses in the assay. For tests run in quarantine in the receiving country, this is probably the largest practical sized arena that could be used. For tests run in the country of the parasitoid's origin, open field tests can be used (Porter *et al.*, 1995).

(C) Adult feeding tests No-choice tests can also be used with predators to assess which prey are used as food. Causton *et al.* (2004) used this approach to determine the host range of the Vedalia beetle, *Rodalia cardinalis* (Mulsant), relative to the native Homoptera of the Galápagos National Park. Alternatively, the goal may be to compare the level of consumption of a prey versus widely distributed foods such as honey dew or pollen. In such cases, it may be useful to note the number of prey of each test species eaten in the presence and absence of the non-prey food.

STRENGTHS

The strength of no-choice tests is that negative results are very robust and provide convincing evidence that a test species is not likely to be used as a field host, provided that the experimental design includes an environment that permits normal behavior of the biological control agent, as evidenced by a positive response to the normal host used as a control. Use of no-choice tests early in the testing sequence provides a strong rationale for classifying unattacked test species as non-hosts. Briese *et al.* (2002a), for example, followed this approach with European insects being screened as biological control agents for *Onopordum* thistles in Australia. Lack of oviposition and adult feeding, especially if coupled with lack of larval feeding, clearly indicate that the test species is not a host. This design guards against the risk of mislabeling a low-ranked host as a non-host, as can happen in choice tests when a preferred host deflects attention away from a less preferred host that might have been attacked if presented alone (see the following section on Choice Tests).

For parasitoids, failure of the adult to oviposit in the appropriate life stage of a test species under no-choice conditions can be taken as fairly solid evidence that the species is not a host if the host is presented on its natural host plant, together with the usual associated kairomones, provided oviposition is observed in suitable controls. The same conclusion can be drawn if oviposition occurs but immature stages fail to develop successfully. Because negative data in no-choice tests are fairly unambiguous, regulators like this type of test. Porter and Alonso (1999) specifically chose a no-choice type test instead of a choice design for testing phorid parasitoids of ants because they wanted to find out whether a native species of ant would be attacked when the target ant was not present, as may happen if the biological control agent's range becomes larger than that of the pest or if the pest's density becomes very low.

WEAKNESSES

Weed biological control practitioners have long felt that weak positive responses to test species sometimes seen in no-choice tests are artificially induced by confinement and the lack of choice. In part, this is accurate – at least in regards the effects of confinement, which brings the agent into close contact with a test species such that important host finding steps may be skipped (since the insect is literally put on the host or very near it), allowing oviposition or adult feeding to occur on species that might not have been detectable in the field. This issue is likely to be particularly important in assessing the host ranges of parasitoids as some acceptable hosts are unfindable by particular parasitoids in nature and hence not really in their host range (e.g., *Pieris virginiensis* Edwards, for *Cotesia glomerata* [L.] [Benson *et al.*, 2003a]). Also, confinement with a non-host or a low ranked host may eventually lead to egg dumping as egg load increases. Without confinement, the agent would be free to disperse and would perhaps find a suitable oviposition site before egg load reached levels leading to egg dumping.

The second complaint, that lack of choice is unnatural, is misleading. Inclusion of choice in the test design is only appropriate if choice is universally present in the field. An insect may in fact not have a choice of hosts (1) if it expands geographically beyond the range of the target pest, (2) if it invades habitats not occupied by the target pest, (3) if the insect is partially out of synchrony with the target pest, or (4) if at the local scale, the target pest is absent for any reason (including biological control itself, chemical control, or even just chance). Thus, there is no reason to say that choice tests are "more natural" or more accurate than no-choice tests. Rather, the system and biology of the organisms in the particular case should dictate which test design is the better model of nature. The researcher, however, must anticipate the full range of these potential settings.

Conversely, even negative responses can be misleading under some circumstances. In particular, a false negative might arise if insects used in the treatment are not in good health or are infertile. This may happen, even with positive responses in the controls, because controls and treatments by design use different individual insects. This problem, however, decreases with suitable replication of both treatments and control or with the use of sequential choice tests.

CURRENT THINKING ON VALUE

Larval development tests No-choice tests are used to measure larval feeding, survival, and development on a potential plant or host. While larval starvation tests were out of favor for weed biocontrol agents in the 1980s and 1990s as too laborious and not focused on the most discriminating stage, their ability to detect the maximum physiological host range has led recently to the test's increased use in weed biological control. For parasitoids and predators, no-choice larval development tests are likely to be even more valuable as this step may be more restrictive than oviposition, particularly for internal hymenopterous parasitoids.

Oviposition tests No-choice oviposition tests have special value because they are able to identify low ranked hosts that can be missed in choice tests. Indeed, the notion that choice tests can "clarify" ambiguous results of no-choice tests in which low ranked species receive ovipositions (see Thompson and Habeck [1989]) is now seen as mistaken. Rather, choice tests can be used to rank hosts if this is desired (Withers, 1999).

For parasitoids, a key feature shaping host ranges will be long-distance attraction to the host via volatiles from the host/plant complex. While choice tests can be run using y-tube olfactometers, there is no advantage to using that design in place of a no-choice design employing olfactometers or wind tunnels. Indeed, misdirected behaviors caused by mixing of volatiles from two or more test species may easily complicate or invalidate test results.

CHOICE TESTS

DESIGN

In choice tests, two or more plant or host species are presented to the biological control agent simultaneously, and thus, the response is a measure of preference between the two options (e.g., McFadyen, 1983; Dunn *et al.*, 1989; Buckingham *et al.*, 1991; Forno *et al.*, 1992; Edwards, 1999). The target species is often, but not always, one of the choices offered. This approach is most commonly used to measure oviposition preferences but can also be used for feeding preferences of adults or even larvae, if these are mobile enough to move between hosts.

A variation on this design is called a sequential choice test (sometimes called a sequential no-choice test; see Chapter 13 [Combs]), in which the natural enemy is exposed to a series of test species, one at a time. Typically, exposure of the agent to a nontarget species begins the test, followed by exposure of the same test insects to the target weed (or host), then after that bout, to a second nontarget weed (or host), and so on. This process is believed to provide a positive control (periodic re-exposure to the target weed verifies continued ability of the tested insects to oviposit or feed) and eliminates the problem of cross contamination of the nontarget species with volatile chemicals from the pest species. A potential flaw of the sequential choice test design is that conditioning induced by experience with the target pest may persist long enough to reduce feeding or oviposition on nontarget species encountered later in the sequence.

While most use of sequential choice tests has been with herbivorous insects, the design has been applied in a few cases to parasitoids. Gilbert and Morrison (1997) used it to assess the host range of the phorid fly *Pseudacteon litoralis* Borgmeier relative to various ant hosts, and Sands and Combs (1999) applied the method to the tachinid *Trichopoda giacomellii* (Blanchard) in tests with Australian pentatomids.

HISTORY OF USE

Weed biocontrol agents Choice tests are still commonly used to estimate the oviposition host ranges of herbivorous insects used for weed biological control (and to a lesser extent, the adult and larval feeding ranges). This design was adopted because practitioners felt that the other alternative – no-choice tests, especially larval starvation tests – was resulting in too many cases in which a species was a host in cage tests but did not seem to be attacked in the field. Oviposition choice tests, it was argued, corresponded better to circumstances in the field because the adult's host seeking was the most discriminating step in the chain of behaviors leading to host use. As a practical matter, these tests allowed some biological control agents to be introduced that might have been rejected based on no-choice tests alone, especially if just no-choice larval feeding data had been considered. Occasionally, workers combined the two, running choice oviposition tests followed by no-choice larval starvation tests (e.g., Dunn *et al.*, 1989; Forno *et al.*, 1992). Use of choice tests with only those species giving positive results in no-choice tests seems to have been done in the mistaken belief that subsequent lack of attack in choice tests would identify which of the positive responses in the no-choice data set were "erroneous" (e.g., Fornasari *et al.*, 1991; Willson and Garcia, 1992).

Currently, this line of reasoning is being re-evaluated by weed biological control practitioners because it has been recognized by some that choice tests are not a good model if, for any reason, the agent is found when and where the target pest is not found (hence, no choice can be made). In such cases (e.g., *Rhinocyllus conicus* [Frölich] on Platte thistle [*Cirsium canescens* Nuttall] in western Nebraska [Louda, 1998]), no-choice tests are a better model for the ecological circumstances the agent is presented with.

Entomophagous species No published examples were found of the use of choice tests for adult or larval feeding by entomophagous predators. Larval feeding and survival by most parasitoids cannot be assessed with this design because larvae have too little mobility, are internal, or both.

Oviposition responses have been measured with choice designs for both parasitoids (Bailey, 1989; Field and Darby, 1991; Keller, 1999; Porter, 2000; Fuester *et al.*, 2001; Babendreier *et al.*, 2003) and predators (Zilahi-Balogh *et al.*, 2002). In some cases, virtually all nontarget species offered were attacked at rates similar to a known host (e.g., *T. brassicae* and various nontarget Lepidoptera [Babendreier *et al.*, 2003]). In this case, it is fairly easy to draw the conclusion that the species is polyphagous. However, it may be useful to present some species to the biological control agent in a no-choice design to determine if volatiles from the target host might have, under choice conditions, contaminated non-target hosts, leading to their attack.

Sometimes in choice tests, the parasitoid shows a strong preference for the target pest (Porter, 2000 for phorid parasitoids of fire ants) or even fails to attack nontarget hosts at all (Fuester *et al.*, 2001, with a tachinid parasitoid of lymantriid moth larvae). These results were interpreted as meaning the parasitoid was strongly focused on the target pest.

With herbivores, the range of hosts that receive ovipositions by a candidate insect has generally been found to get smaller under choice conditions in which the choice includes the target pest. The same pattern was observed with the predatory beetle *L. nigrinus* when offered nontarget species either separately or together with the target pest, hemlock woolly adelgid (Zilahi-Balogh *et al.*, 2002). However, for several studies of parasitoids, the opposite pattern occurred, and the number of species accepted for oviposition, or the level of attack on less preferred hosts, increased rather than decreased under choice conditions (Bailey, 1989; Field and Darby, 1991; Barratt *et al.*, 1997; Keller, 1999). This suggests that for parasitoids the effect of choice conditions may primarily be to stimulate the parasitoid to attack (by providing kairomones from the target pest), causing attacks on hosts not themselves able to stimulate oviposition. For parasitoids, no published examples were found of attack on a nontarget species in no-choice tests where attack disappeared in choice tests containing the preferred host. If further examples demonstrate this pattern to be generally true, then the risk that choice oviposition tests with parasitoids would lead to false negatives would be smaller than it appears to be for herbivore responses to plants. Given that to be the case, negative data in choice oviposition tests would be a more robust indication of safety to nontarget species. In fact, there may be cause to worry that use of a choice-design will lead to false positive ovipositions. Thus, for parasitoids, it might be reasonable to use choice designs to screen a large number of test host species, following up with no-choice tests for all species receiving ovipositions in choice tests (to detect false positives).

STRENGTHS

The choice design is well suited to reveal if the agent shows a preference among potential host species (typically, the choice presented is between the target pest and one or several nontarget species). This design also allows a more rapid examination of many species of potential hosts than is possible if each must be studied separately. Finally, the rank order of preference among hosts can be established with removal of the most preferred host, followed with repetition of the test until all hosts have been ranked. This approach is called "choice minus target" (or sometimes "choice minus control') and is discussed below under the heading Preference Ranking Test.

WEAKNESSES

The weakness of this design is that preference for host A over B, when the two are presented together, is often erroneously interpreted to mean that B is not a host. (To illustrate: a child presented with broccoli and a pizza will almost certainly eat the pizza only, but this should not be taken to mean that humans do not eat broccoli. A very hungry child presented only with broccoli will eat it, eventually.) For the results of a choice oviposition test to be predictive of field events, (1) the agent must experience the choice in the field – that is, the nontarget (low rank) host must not be the only possible host encountered or (2) the nontarget (low rank) host must be so non-preferred that even agents deprived of their preferred hosts for considerable periods will keep searching rather than attack the low ranked species. Since these conditions may not always be met, inferring that a species not attacked in a choice oviposition test is not a host will lead to some unpredicted impacts. To understand if choice tests are appropriate, one must look at the options likely to be available to a foraging individual of the released species.

For parasitoids, it may be argued that choice would rarely occur in the experience of individual insects because they are most likely to encounter potential hosts one species at a time and it may not be the most preferred species. Whether or not parasitoids live in a "multiple-choice world" would be a valuable research area.

Several examples have been noted in which preference in laboratory tests for the target pest proved not to be predictive of safety in the field for the non-preferred native or crop species. *Rhinocyllus conicus* has been found feeding extensively on Platte thistle (Louda, 1998) despite a preference in laboratory tests for the target pest, musk thistle (*Carduus nutans* L.) (Arnett and Louda, 2002). Similarly, the invasive weevil *Larinus planus* (F.) (since used as a biological control agent) is now attacking Tracy's thistle (*Cirsium undulatum* [Nutt.] Spreng. var. *tracyi* [Rydb.]) in Colorado (Louda and O'Brien, 2002) despite predictions that it would not do so (McClay, 1990).

CURRENT THINKING ON VALUE

Some biological control practitioners still think that choice tests are useful as a means to determine if positive results found in no-choice tests are "real" by seeing if they still occur in a choice design that includes the target pest (presumably a highly preferred species) (Briese *et al.*, 2002a). This seems, however, to be a basic misunderstanding of the biology being studied (see the *Weaknesses* section, above). Rather, the value of choice tests is as a means to construct a rank order of preference within the list of possible hosts (using choice minus target pest tests, as described below). All members of the ranked host list should, however, be considered hosts unless larval starvation tests show that they do not complete development or continuation tests (see below) show that the species is so poor a host that population growth rate is below replacement and the agent dies out after a few generations.

The value of this design may be greater for parasitoids than for herbivores if it proves to be generally true that choice design tends to expand rather than shrink the set of test species attacked (see above). In such a case, negative data for nontarget species for tests with parasitoids may be a more robust indication of safety than is the case for herbivores. For plants, the predominant risk of choice tests is false negatives. For parasitoids, this risk seems lower, and there may even be a significant risk of false positives. The importance of these errors needs to be assessed by comparison of such results to data from no-choice tests for a series of parasitoid species.

OPEN FIELD TESTS

DESIGN

Open field tests have largely been limited to tests of herbivore oviposition on plants. These assays are uncaged tests run outdoors, either in a garden or in a natural stand of the target weed, where potted plants of the nontarget test species are interspersed among the target plants (Clement and Sobhian, 1991; Briese *et al.*, 1995; Clement and Cristofaro, 1995; Briese, 1999). The agent is either present as a natural population or additional individuals are released to augment the natural background density. The outcome of the test is usually measured as the number of eggs laid on each test plant.

HISTORY OF USE

Weed biocontrol agents The open field test was developed at the end of the 1980s (e.g., Clement and Sobhian, 1991) on the belief that removing test plants and insects from cages and letting their interactions occur in an open space eliminated erroneous results that occurred when test insects were denied the option to leave the test arena. (In cages, with emigration denied, oviposition sometimes occurs on plants believed to not really be hosts, or even on the cage itself.) Open field tests are typically described as being "more natural" than cage tests. An issue in tests with this design is effects caused by the pattern and sizes of the patches of the various test plants in particular tests. A variation of this test, called a "two-phase open-field test," has been developed to determine what the test insects would do if the target weed's population were suddenly not available (Briese *et al.*, 2002b).

Entomophagous species Use of open field tests as done for herbivores depends critically on being able to move test plants native to the region of proposed agent introduction to the agent's country of origin. This is often possible if the test plants have already been moved, for economic or ornamental use, into the country where the tests are to be run or if plants are released from quarantine only in pots and care is taken not to allow seeds or plant fragments to escape.

For parasitoids and predators, however, it is typically impossible to move the nontarget test insects into the country of origin of the agent because these are usually herbivorous insects and might become pests. Consequently, the only way to employ open field tests in the areas of origin of parasitoids and predators proposed for introduction is to assess attack on local species that are phylogenetically close to the species of concern in the proposed area of introduction. This was done, for example, by Porter *et al.* (1995), who exposed a series of local species of ants in Brazil to phorid parasitoids. By this means, data were obtained suggesting that these flies were host specific at least to the genus level. Similarly, Fuester *et al.* (2001) collected 54 species of European caterpillars in 11 families to assess the frequency of parasitism by the tachinid *A. samarensis*, a proposed biological control agent for the gypsy moth, *Lymantria dispar* (L.). Both of these projects suggested a high level of specificity for the parasitoids under study. However, the inability to test the actual native species potentially at risk in the area where the agent is to be introduced remains an important limitation.

STRENGTHS

In open field tests, test insects do not experience any unnatural influences that might alter their behavioral responses to potential hosts, such as altered light quality within cages, increased egg loads, or stimulation or repression of their sensitivity to plant chemicals that might come from forced confinement on or near either the test plant (stimulation) or various non-target plants (sources of potential deterrents).

WEAKNESSES

Open field tests can only be done in the native range of the biological control agent that is being studied, as quarantine considerations prevent the test from being done in the area into which the insect is proposed for introduction. Technical considerations that have been mentioned as potential defects are the relative balance of numbers of test plants and the target weed (likely to be strongly tilted toward the pest species) and the density of the test insects (likely to be low) (Briese, 1999). But the most important problem for these tests is the same as for choice tests run in cages (since open field tests, as used, have all been choice tests): namely, that what is measured is preference. Thus, like caged choice tests, open field tests are set up to miss low ranking hosts precisely because the preferred species, the target weed, is always present.

In theory, this might be corrected in the context of weed biological control if trials were run as no-choice tests. Groups of potted tests plants of just a single species (either the target pest or one of a series of nontarget species) could be placed outdoors and test insects released onto or near by such test plants. Such tests would have to be made at sites isolated from stands of the target weed to avoid contamination by individuals of the test insect arising from wild plants and having a different set of past host experiences. However, there are no published examples of open field tests set up as no-choice tests. A partial approach to creating such conditions is found in a test termed a "two-phase open-field test" (Briese *et al.*, 2002b). Steps in the such a test are (1) creating a common garden plot containing the target weed and various nontarget test plants, (2) allowing the candidate biological control agents to colonize the plot, (3) taking data on the agents' feeding and oviposition, and then (4) killing the target weed plants. This forces the agents to switch and accept lower ranked hosts, emigrate, or die. When this approach was used by Briese *et al.* (2002b) for four candidate species attacking the weed *Heliotropium amplexicaule* Vahl, a pest in Australia, it was found that three agents either left or died, but one (an undescribed flea beetle, *Longitarsus* sp.), switched to feeding on the nontarget species *Heliotropium arborescens* L.

CURRENT THINKING ON VALUE

Open field tests have not become widely used (Briese, 1999), in part because they must be done in the country of origin of the agent, in part because of the potential quarantine problems of moving test organisms to that region, and in part because they are seen as a final step, not a first step (and thus, are sometimes not needed to make a regulatory decision at that stage of the review). Practical difficulties that frequently result in poor quality test results are common. There may be too few test insects, for example, at the sites. Such considerations have discouraged the use of these tests in weed biological control.

Applying these tests to insect targets (as opposed to plants) is even less feasible because of quarantine concerns with the desired test species. Conceivably, simulated open field tests could be constructed using walk-in cages within naturally lighted, quarantine greenhouses, which would provide large spaces for natural insect behavior to occur and ample opportunity for test insects to leave the system if they so desired. For entomophagous species, use of open field tests would be further complicated in some cases by the mobility of the nontarget species used in the test (such as for active bugs or caterpillars). This would not be an issue for some groups such as scales, aphids, whiteflies, and psyllids, or if the stage attacked was relatively immobile (eggs, pupae, or very small larvae).

CONTINUATION AND OOGENESIS TESTS

DESIGN

These tests focus on the suitability of the host to support the test insect's population over the long term. Oogenesis tests determine whether the host is nutritionally adequate to promote the agent's egg development. Continuation tests measure whether the host can support a population of the agent indefinitely, with an growth rate greater than replacement. These things must happen if the agent's population is to survive with no other resources. These tests are no-choice in design, and combine the oviposition, feeding, and survival responses all together.

HISTORY OF USE

Neither of these tests is widely used, and all examples found were for herbivorous, not entomophagous, insects. Kok *et al.* (1979) included oogenesis tests in their study of the weevil *Ceutorhynchus trimaculatus* F. They showed that this species was able to develop eggs when it fed on various thistles or artichoke but not on safflower.

Continuation tests were run by Buckingham *et al.* (1989), who found that a population of the fly *Hydrellia pakistanae* Deonier died out within eight generations if reared exclusively on the nontarget pondweed *Potamogeton crispus* L., suggesting that this species is not a satisfactory host even though the insect can survive on it and produce offspring for several generations in decreasing numbers. The value of continuation tests to host specificity assessment has been discussed by Day (1999).

In some cases, experience with *de facto* continuation tests in one country may provide information valuable in assessing risk in another. In South Africa, the mirid bug *Eccritotarus catarinensis* (Carvalho) was released for control of waterhyacinth (*Eichhornia cras*sipes [Mart.] Solms-Laub). This bug was found in laboratory assays to feed on pickerelweed (*Pontederia cordata* L.), a nonnative invader in South Africa. The mirid bug failed to establish persistent breeding populations on pickerelweed, both when released directly onto pickerelweed plants in cages and at sites where stands of waterhyacinth (with established populations of the bug) were close to stands of pickerelweed (Coetzee *et al.*, 2003). These results constitute a field continuation test and show that, if the bug were to be introduced to the United States (where waterhyacinth is an invasive pest but pickerelweed is a native plant), it would be unlikely to establish itself on pickerelweed.

STRENGTHS

These tests are a strong complement to larval starvation tests because they indicate the degree of risk that isolated populations of nontarget species might face if their habitat were to be invaded by the biological control agent, in the absence of the target pest. Failure of the population of an agent to survive for multiple generations on the target pest is a robust indication that in the field the agent could not threaten native species at sites isolated from the target pest.

WEAKNESSES

These approaches, especially the continuation test, are expensive to run as they extend for a longer time than other commonly used tests. Also, these tests may fail to predict impacts on nontarget species that may occur in cases where the agent's population is sustained by the target pest and spills over onto the nontarget species. In this circumstance, even though the nontarget species did not support the agent's population, it might decline in its presence.

CURRENT THINKING ON VALUE

These tests are considered to be helpful in assessing risks to low ranked hosts. Another potential value of such multigenerational tests is to see if adaptation occurs between a parasitoid and a new host, such that progeny reared from a novel host accept that host more readily than did the parental generation, or have better growth or survival in it.

PREFERENCE RANKING TEST (CHOICE MINUS TARGET)

DESIGN

This test reveals the relative ranking of a particular host (within a test list) for a candidate biological control agent, from most to least attractive (usually for oviposition). In ecological studies, for example, it is of interest to understand the relative degree of attractiveness of various plants. It should be noted, however, that a lower ranking but more abundant plant might be a more important host to an agent – in terms of population level consequences – than a more preferred but scarcer host.

With herbivorous insects, rankings are inferred by presenting the insect with a group of plants all together in a cage, observing the species on which most eggs are laid, removing that species, and repeating the test. This process continues until all plants have been ranked. In practice, if the test list is very long, each test may contain only a random subset of the test plants. This also has the advantage of being more likely to average out any distorting effects introduced by one strongly stimulating or strongly inhibiting test species, whose presence in some subsets may change the insect's response to other plants in that grouping. The rankings within these subsets must then be fused into one master ranking.

A factor affecting the outcomes of such tests (indeed of all host preference tests, regardless of design) is the past experiences of the test insects with other hosts, especially the target pest. This important issue is discussed in Chapter 5.

HISTORY OF USE

The idea that an insect's hosts can be arranged in a hierarchy from most to least preferred was developed in basic studies of insect-plant interactions (e.g., Wiklund, 1975, 1981; Thompson, 1988; Jallow and Zalucki, 1996) and was later incorporated into biological weed control host range estimation as a variation on choice tests. The concept applies as well to ranking the hosts of a parasitoid or the prey of a predator, but published examples all concerned herbivorous insect assessment of plants.

An early step in this direction was simply to repeat a choice test with the target pest species omitted and compare the two data sets. Hill *et al.* (1995), for example, made this comparison for oviposition by the moth *Agonopterix ulicetella* (Stainton) (Oecophoridae), a gorse (*Ulex europaeus* L.) insect. All test species were present in the first series, while in the second, gorse was removed. They found that removing gorse from the test resulted in increased oviposition on five of nine nontarget species tested (and reduced oviposition on four others, but these were mainly switches of trivial degree, such as from 1 to 0). Interestingly, these researchers also included a test in which test insects had no previous exposure to gorse. These insects also oviposited on a wider range of plant species (compared to tests including gorse), some of which were not attacked by individuals with prior exposure to gorse. Only five studies (Peschken and Harris, 1975; DeLoach *et al.*, 1976; Wapshere and Kirk, 1977; Cordo, 1985; and Withers *et al.* 1999) are mentioned in Edwards' (1999) review as having included both choice tests with the target weed present and with the target weed removed. Actual use of this approach is likely to be more common (e.g., Hill and Gourlay, 2000).

An extension of this process is then to specifically identify the first and second most preferred hosts in two rounds of testing, in which the most preferred species in round one is removed and the test continued with fresh plants (but the same insects) to identify the second-most preferred host. This was the approach taken by Solarz and Newman (1996) in establishing the host preferences of the native watermilfoil specialist weevil *Euhrychiopsis lecontei* (Dietz). This study also found that rearing the test insects on the target weed (Eurasian watermilfoil) induced a preference for this species in subsequent tests (see Chapter 5 on factors affecting tests). However, it should be noted that it is often nearly impossible to avoid doing so, given constraints of what the agent can be effectively reared on.

Marohasy (1998) later recommended that this process be further elaborated so that all hosts could be ranked by repeating the test multiple times, removing the most preferred species in each run, until only one host (the least preferred species) remains. Edwards (1999) has reviewed past choice tests and lists those studies that have run choice-minus-target experiments. So far few biological control studies have followed Marohasy's (1998) strategy for ranking hosts.

STRENGTHS

Preference ranking tests show in what order plants (or for parasitoids, hosts) would be accepted if an insect were aware of two or more potential hosts at the same time and place. All acceptable hosts, however, are in the host range, even the least preferred. Consequently, in practical terms this test design has limited value in countries that have a highly risk aversive stance on biological control.

WEAKNESSES

Preference ranking tests, like choice tests in general, have been misinterpreted to imply that lower ranked hosts are non-hosts. This mistake has now been pointed out (Edwards, 1999). Preference ranking tests are also time consuming and only justified if there is a clear need to learn where particular species rank as hosts. Perhaps for these reasons, this procedure has not been widely adopted.

CURRENT THINKING ON VALUE

Preference ranking tests seem of greater value in the understanding the host plant choices of native insects than in estimating the safety of species proposed for introduction as biological control agents because, by definition, all the hosts being ranked are inside the host range. The added knowledge of their precise place in the preference hierarchy is not information that can be used to change the release/reject decision for which host specificity data are being collected. The introduction of an insect for which a native plant is a very low ranked host might be acceptable if the pest weed caused great ecological or economic damage as the lesser of two evils – but this is a political decision. A low ranked host remains a host.

TESTS FOR TIME- AND BEHAVIOR-DEPENDENT EFFECTS

One of the directions in which host range testing has been moving in recent years for weed biological control projects is to pay more attention to physiological factors that can alter the test insect's response to plant species. (For a full discussion of these influences, see Chapter 5.) Among these factors are the age of the insect, its egg load, its experiential history, such as the insect's past contacts with stimulatory or inhibitory chemicals on plants, and the time that has passed since its last feeding or oviposition bout (leading to time-dependent changes in behavior). Study of these factors leads to new ways of testing insects. For example, to determine if there is an effect of age, both "young" and "old" insects must be considered in tests as distinct treatments. Similarly, if the effect of experience is to be understood, both naïve insects and those with experience with the target species must be tested separately. These issues have long been recognized as affecting oviposition choices of parasitoids and are explored in Chapter 5.

Particular attention has been paid to time-dependent influences (amount of time since the last feeding or oviposition bout) and how these would affect the outcomes seen in both choice and no-choice tests (Browne and Withers, 2002). Higher ranked hosts are likely to be eaten or used for oviposition earlier than lower ranked hosts. On higher ranked hosts, meals are likely to be larger and egg batches deposited more frequently. Since the refractory phase (the period after a bout on a preferred host during which a less preferred host elicits no response) can vary from minutes to days, the details of each agent's biology will influence the nature of responses observed in tests. If a species remains refractory to a less preferred host for a long time (relative to the length of the test or the period between contacts with the preferred host), then one predicts that the less preferred hosts will not elicit any response and be incorrectly scored as a non-host. Also, in no-choice tests, low ranked hosts will eventually be used if the test lasts long enough. Such responses may be viewed as false positives if one assumes that the target, preferred host would be contacted in the field before the less preferred host is used. However, if one projects that the biological control agent might enter a geographic or ecological zone where the more preferred hosts are not present, or that a partial asynchrony of the agent with its target hosts can cause a temporary lack of the suitable life stage of the preferred host, then this outcome in laboratory tests would not be a false positive, but would accurately foretell exploitation of a low ranked host.

POST-RELEASE VALIDATION OF PREDICTIONS

A final necessary step in the process of developing effective testing methods is to score the accuracy of predictions by post-release evaluations of realized field host ranges. In general, there have been only selective, partial attempts in this direction. One set of studies is that in which an agent is discovered or suspected of attacking non-target species in the field and then studied more intensively (e.g., Louda, 1998; Louda and O'Brien, 2002; Benson *et al.*, 2003b). These studies, however, begin with prior knowledge of likely impact and therefore do not collectively estimate the average outcome.

More comprehensive reviews of whole sets of cases are needed. Sources of data, however, for such a wide range of cases are hard to come by. Pemberton (2000) reviewed the literature on weed biological control agents in North America, Hawaii, and the Caribbean and found that most nontarget species reported in the literature as fed on by biological control agents were congeners of the target weed. Willis *et al.* (2003) described a preliminary assessment of the impacts of weed biological control agents on nontarget plants in Australia; however, these authors' ability to assess the robustness of original predictions was reduced by lack of access to unpublished records of quarantine laboratories that did much of the testing. Only published records could be evaluated. Several studies currently underway in New Zealand and Australia are expected to provide additional much-needed post-release evaluations of realized versus predicted host ranges.

CONCLUSION

From the previous discussion, we can draw several conclusions. First, it is clear that tests should model the ecological contexts in which agents will interact with potential hosts. In many cases, this will be a no-choice context, making choice tests a less useful means of predicting outcomes. Second, unlike for herbivorous insects interacting with potential host plants, in which the discriminatory step is most often oviposition rather than larval feeding, the reverse may be the case for parasitoids. For parasitoids, larval survival in the host may be more discriminatory than adult oviposition. Third, oviposition tests should strive to include host finding as well as host acceptance in assays by using larger arenas, with natural host plants and air circulation. Fourth, use of naïve rather than experienced adults will better reveal the breadth of the potential host range. Fifth, open field tests and host preference tests seem of limited use for work with entomophagous insects: the former because of quarantine issues and the latter because a low host preference increasingly seems an inadequate reason for considering a native species as not a host or as not at risk. We expect there will be a need to reassess these tentative conclusions in the future as more studies seek to estimate host ranges of entomophagous insects, providing more data sets on responses in alternative experimental designs.

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CHAPTER 8. ESTIMATING THE HOST RANGE OF A THRIPS PARASITOID

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BACKGROUND

New Zealand has recently adopted new legislation (The Hazardous Substances and New Organisms Act [HSNO] of 1996) governing the importation and release of new organisms, including insect biological control agents. The primary function of HSNO is to protect the environment, people, and communities from potential adverse effects of hazardous substances or new organisms. The introduction of the HSNO act has created a number of significant changes in the process for introduction of new biological control agents. For example, the introduction of a new organism requires a thorough assessment of possible risks, costs, and benefits, and there is more emphasis on consultation with concerned parties and potential negative environmental impacts on indigenous non-target hosts.

One of the first new organisms approved for release into New Zealand after this legislation was passed was *Thripobius semiluteus* Boucek (Hymenoptera: Eulophidae), a parasitoid of the greenhouse thrips, *Heliothrips haemorrhoidalis* Bouché. Before its release in New Zealand, an extensive research program was conducted to determine the host range of *T. semiluteus* and to 'demonstrate nil or negligible environmental impacts on New Zealand's flora, fauna, environment, and indigenous culture' as required under HSNO.

Some aspects of the information developed to support an application for the introduction of *T. semiluteus* are presented in this chapter. We first describe the ecology and biology of the pest and the proposed agent, followed by a description of the fauna in New Zealand potentially at risk from the introduction. Our principal focus in this chapter is to evaluate the assumptions and logic that guided our testing program, technical aspects of the methods used, and our interpretation of the results obtained.

DESCRIPTION OF PEST INVASION AND PROBLEM

Heliothrips haemorrhoidalis (Thysanoptera: Thripidae; subfamily Panchaetothripinae), previous synonym *Thrips haemorrhoidalis,* is a ubiquitous species and has been recorded in 41 countries (Rivnay, 1935; Bodenheimer, 1951; Mound and Walker, 1982; Gerson, 1983; Ananthakrishnan, 1984; Goodall *et al.,* 1987; Beattie and Jiang, 1990; Kudô, 1992; Phillips, 1992; Dupont, 1993; Childers and Frantz, 1994; Phillips *et al.,* 1995). It is believed to have originated in South America in the Neotropics but is now widespread in tropical, subtropical, and temperate areas (Mound and Walker, 1982). *Heliothrips haemorrhoidalis* was first recorded in New Zealand in the 1930s and is presumed to have been accidentally introduced on imported plant material. It is abundant outdoors in the subtropical to temperate North Island and is found as far south as Christchurch in the more temperate South Island (latitude range in New Zealand of 36° to 44°) (Mound and Walker, 1982).

Heliothrips haemorrhoidalis is polyphagous and has been recorded on more than 60 species of plants worldwide (Ananthakrishnan, 1984) and over 30 in New Zealand (Spiller *et al.,* 1982). Records from New Zealand are mostly restricted to subtropical fruit trees and cultivated garden trees and shrubs, with just two adults recorded from native forest areas (Mound and Walker, 1982). However, Martin and Mound (2004) have recently recorded small numbers of *H. haemorrhoidalis* in disturbed native forest and forest margins.

Heliothrips haemorrhoidalis is uniparental, with only females being produced. It is a significant economic pest in the subtropics and warmer temperate areas, where it occurs outdoors in very large numbers and can reproduce year round with several overlapping generations per year. In New Zealand, it is a significant pest on a number of commercial horticultural crops, including citrus and avocado (Figure 1). It has also been recorded as damaging nursery stock of two important forestry species, *Pinus radiata* D. Don and *Pseudotsuga menziesii* (Mirbel) Franco (Zondag, 1977). *Heliothrips haemorrhoidalis* has no effective natural enemies in New Zealand, and therefore growers rely on chemical or cultural control.

DESCRIPTION OF AGENT PROPOSED FOR INTRODUCTION

Thripobius semiluteus (Figure 2) was described in 1976, but earlier records exist of what is believed to be the same species based on specimens collected in Africa in 1931 and referred to as *Thripoctenus* (= *Ceranisus*) sp. This parasitoid has been recorded from tropical and subtropical areas of Africa, Asia, South America, and Australia. Research programs to determine the most efficacious and host-specific parasitoid for introduction against *H. haemorrhoidalis* have led to the introduction of *T. semiluteus* into California, Florida, and Hawaii (in the United States), as well as into Japan and Israel (Boucek, 1976; Hessein and McMurtry, 1988; LaSalle and McMurtry, 1989; Beattie and Jiang, 1990).

This parasitoid is a solitary koinobiont endoparasitoid that has been recorded from five species of thrips all within the subfamily Panchaetothripinae. It is uniparental (Loomans and

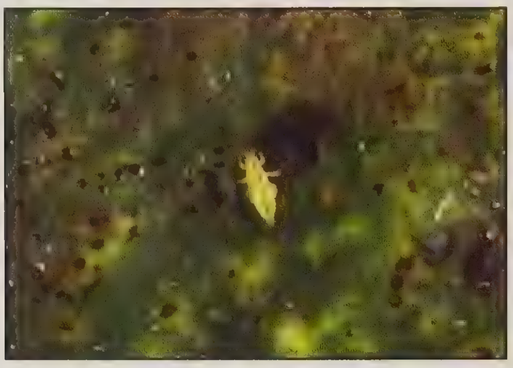

Figure 1. *Heliothrips haemorrhoidalis* adult and damage on avocado. Photo: M. Henderson. (UGA1295002)

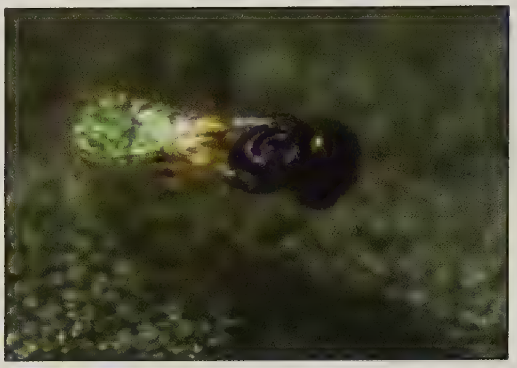

Figure 2. *Thripobius semiluteus* adult. Photo: D. Allan. (UGA1295003)

van Lenteren, 1995), and females oviposit single eggs into either first or second instar thrips (McMurtry *et al.*, 1991). When searching for hosts, the parasitoid walks in a slow sideways motion over the plant surface (Loomans and van Lenteren, 1995) and, on finding a suitable host, the female achieves oviposition in only 1-3 seconds before moving on to search for another host (Froud, personal observation). The parasitoid larva remains inside its host until the late second instar (just prior to the pre-pupal stage), when the host dies. The parasitoid then transforms into a black pupa, which remains cemented to the plant surface (where the host moves prior to pupation) until emergence of the adult parasitoid (Loomans and van Lenteren, 1995). The generation time for *T. semiluteus* at 23°C is 21 days, of which 11 days is spent in the pupal stage (Froud and Stevens, 1997). The adult longevity of *T. semiluteus* at 23°C is only 2.9 days, with the majority of eggs being laid on the second day following adult emergence (Froud and Stevens, 1997).

Source of agent *Thripobius semiluteus* was imported into the HortResearch insect quarantine facility at the Mt. Albert Research Centre, Auckland, New Zealand, in 1995 from F.A.R. Inc. Insectaries in Corona, California, USA. The *T. semiluteus* population in California was originally collected from parasitized *H. haemorrhoidalis* in Australia and Brazil. A subsample of parasitoids was checked by Frances Mafile'o, HortResearch, confirming that the shipment was free of insect pathogenic micro-organisms. Species identification and examination for hyperparasitoids were done by Dr. J. Berry, Landcare Research, Auckland, New Zealand, and voucher specimens were deposited with the New Zealand Arthropod Collection (NZAC), Landcare Research, Auckland, New Zealand.

Hosts in the native range of agent *Thripobius semiluteus* has been recorded as a parasitoid of five species of Thripidae, all in the subfamily Panchaetothripinae. LaSalle and McMurtry (1989) list *Brachyurothrips anomalus* Bagnall, *Panchaetothrips indicus* Bagnall, and *H. haemorrhoidalis* as hosts. Loomans and van Lenteren (1995) identified *Selenothrips rubrocinctus* Giard and *Hercinothrips femoralis* Reuter as additional hosts for *T. semiluteus*; however, parasitism of *H. femoralis* was only observed under laboratory conditions, never in the field. Of these five species of thrips, only *H. haemorrhoidalis* is known to be present in New Zealand.

THE RECEIVING LOCATION

Description of Fauna in Area of Proposed Agent Introduction

The thrips fauna of New Zealand is well documented (Mound and Walker, 1982, 1984) and includes a number of indigenous and invasive species. *Heliothrips haemorrhoidalis* is a member of the Thripidae, which is represented in New Zealand by two subfamilies, the Thripinae and Panchaetothripinae. *Heliothrips haemorrhoidalis* is in the Panchaetothripinae and is one of four species, in four genera, of this subfamily found in New Zealand (*H. haemorrhoidalis, Hercinothrips bicinctus* Bagnall, *Parthenothrips dracaenae* Heeger, and *Sigmothrips aotearoana* Ward). The first three are exotic pest (or potential pest) species. The last species, *S. aotearoana,* is a native thrips. In the other subfamily, Thripinae, there are 43 species in New Zealand, of which 18 are indigenous. Of the remaining 25 species, one – *Sericothrips staphylinus* Haliday – is an introduced weed biological control agent.

Local Species of Value as Biological Control Agents

Of the thrips species present in New Zealand, only one is considered of value as a biological control agent. *Sericothrips staphylinus* was introduced as a biological control agent to control gorse (*Ulex europaeus* L.), a severe weed of agricultural and natural lands in New Zealand. This thrips was introduced to New Zealand in 1989 from Europe. Later, new introductions were made with material from Hawaii (originally from Portugal). Due to the presumed restriction of *T. semiluteus* to thrips in the subfamily Panchaetothripinae, this Thripinae species was not considered a likely target for parasitism.

Displacement of native parasitoids and predators through the introduction of *T. semiluteus* was also considered as part of our studies. There are few natural enemies of thrips in New Zealand. Apart from one record of *Ceranisus* sp. from *H. haemorrhoidalis,* no other larval parasitoids are known for *H. haemorrhoidalis* in New Zealand (Mound and Walker, 1982; D. Steven, IPM Research, Auckland, New Zealand, unpublished data). A species of *Megaphragma* has also been recorded from *H. haemorrhoidalis* eggs; however, research has shown that it is not an effective parasitoid of *H. haemorrhoidalis* in New Zealand (Chhagan, 2002; D. Steven, IPM Research, Auckland, New Zealand, unpublished data). Several predators attack thrips in New Zealand, including three small solitary wasps in the genus *Spilomena.* These wasps capture thrips to feed to their larvae. One species of *Spilomena* has been recorded collecting large numbers of *H. haemorrhoidalis* larvae, but had little impact on thrips numbers (Mound and Walker, 1982). Two anthocorids (Homoptera), *Cardiastethus consors* White and *Cardiastethus poweri* White, also attack thrips in New Zealand (Lewis, 1973), as do some dipteran larvae and vertebrates. Also, several predatory thrips in the family Aeolothripidae and some ectoparasitic mites in the genus *Adactylidium* (Pyemotidae) can attack *H. haemorrhoidalis* (Mound and Walker, 1982). An *Entomophthora* species of fungus has also been recorded as infecting some species of thrips in New Zealand. None of these natural enemies, however, is effective at reducing *H. haemorrhoidalis* populations, and due to their generalist nature these species are unlikely to be displaced by the introduction of a larval parasitoid.

LOCAL SPECIES OF MARKED CONSERVATION VALUE

While there are no endangered or charismatic species that could be harmed by this introduction, some New Zealand thrips have unique interest as local products of evolution. *Sigmothrips aotearoana* was first described in 1970. It is a monobasic genus endemic to New Zealand and has only been collected from native forests. Only females have been observed. Adults have been collected in all months and are often found together with larvae in association with visible damage (Mound and Walker, 1982). Little else is known of the biology of S. *aotearoana,* other than that it lives mostly on seedlings of the indigenous plants *Coprosma* spp. and *Geniostoma ligustrifolium* Cheeseman and pupates in the soil (Mound and Walker, 1982; Froud, 1997). This is in contrast to *H. haemorrhoidalis,* which pupates on the host plant. The distribution of *S. aotearoana* in New Zealand is more extensive than that of *H. haemorrhoidalis* and includes a population in Southland (46° S, 169° E) (Mound and Walker, 1982). The Southland population is well beyond the known and potential range of *H. haemorrhoidalis* and *T. semiluteus,* respectively (43° S, 172° E) (Froud and Stevens, 1997).

THE TESTING PLAN: ANALYSIS OF METHODS

SPECIES LIST FOR HOST RANGE TESTING

Only members of the Panchaetothripinae seem likely to be within the host range of *T. semiluteus* in New Zealand because *T. semiluteus* has never been recorded from any species of Thripinae, despite rearing of these thrips to detect parasitism in areas where *T. semiluteus* occurs (LaSalle and McMurtry, 1989; Beattie and Jiang, 1990; Loomans and van Lenteren, 1995). Within the Panchaetothripinae, only two non-target species were selected for host range testing – *S. aotearoana* and *H. bicinctus* – because the only other member of the subfamily found in New Zealand – *P. dracaenae* – is an introduced pest. The target species – *H. haemorrhoidalis* – was included in choice tests and used as a control for comparison in no-choice tests.

Sigmothrips aotearoana was selected due to its conservation value as a monobasic genus and as an important member of New Zealand's endemic biodiversity. *Hercinothrips bicinctus* was subsequently included in the test list following low levels of parasitism of *S. aotearoana* by *T. semiluteus* in initial host range tests. Exposing the closely related *H. bicinctus* to *T. semiluteus* under the same laboratory conditions and experimental methods was done to assess the possibility that the low level of parasitism of *S. aotearoana* found in our tests was an artefact of confinement or imperfect host recognition (Michaud and Mackauer, 1995). *Hercinothrips bicinctus* is present in Australia, where it is sympatric with both *T. semiluteus* and *H. haemorrhoidalis,* but it has not been recorded as a host for *T. semiluteu*s (pers. comm. A. Loomans, Wageningen, The Netherlands; M. Steiner, NSW Department of Agriculture, Australia; J. Noyes, Natural History Museum, U.K.).

GENERAL DESCRIPTION AND JUSTIFICATION OF TESTS

Both no-choice and choice host tests were carried out to estimate the host range of *T. semiluteus.* No-choice tests were undertaken to determine if *T. semiluteus* would use non-target species in the absence of *H. haemorrhoidalis.* Choice tests were undertaken to determine if *T. semiluteus* would show a preference for *H. haemorrhoidalis* (if two species were available at the same time and place) and to determine the likelihood of non-target oviposition in a confined space where host cues could be commingled or at least spatially very close.

We also studied the life history and population dynamics of *S. aotearoana* and *H. haemorrhoidalis* to determine the likelihood of *T. semiluteus* encountering *S. aotearoana* and sustaining viable populations on this non-target host in the New Zealand environment.

TEST #1: HOST TESTING OF *T. SEMILUTEUS* AGAINST *S. AOTEAROANA* AND *H. BICINCTUS*

The goal of this test was to determine the ability of *T. semiluteus* to oviposit and successfully develop in *S. aotearoana* and *H. bicinctus.*

Methods for Test #1 Parasitoids used in tests were reared on *H. haemorrhoidalis* larvae on partially ripe lemons in sealed containers in a small, sealed room within the Insect Quarantine facility at 23C, 65-75% R.H., and a 16:8 L:D photoperiod. All host testing experiments were conducted within the same room under the same conditions. Five naive female parasitoids of a known age (24-48 hours after emergence) were introduced into a test arena (a clear, 3.5 liter plastic container, measuring 20 x 15 x 20 cm, with fine mesh ventilation) for both the choice and no-choice tests. Each comparison was replicated five times. *Heliothrips haemorrhoidalis* larvae were obtained from a colony reared in the laboratory on lemons. *Sigmothrips aotearoana* were reared in the laboratory from field-collected adults from *G. ligustrifolium* seedlings, and *H. bicinctus* larvae were obtained from field collections from Kapok vine, *Araujia sericifera* Brot. During exposure to parasitoids, *H. haemorrhoidalis* larvae were placed on a single lemon and the other test species were placed on their respective host plants (as described below). Thrips were transferred using a fine camelhair brush that was cleaned in 95% ethanol between species.

In no-choice tests, a group of five *T. semiluteus* females was exposed to either to (1) 50 first instars of *S. aotearoana* on a *Coprosma robusta* Raoul seedling (a common host plant of *S. aotearoana* that was readily available and could be kept alive during the experiment in a small flask stoppered with cotton wool and covered with Parafilm™—see Figure 3) or to (2) 50 first instars of *H. haemorrhoidalis* on an unripe lemon in the test arena. In choice tests, five *T. semiluteus* females were provided with 50 first instars of *S. aotearoana* and 50 of *H. haemorrhoidalis,* presented as described above. For both tests, adult parasitoids were removed after 24 hours and thrips larvae were separated by species and placed in containers to complete development. After two weeks, damp vermiculite was placed in the test containers with *S. aotearoana* to support pupation.

The methodology used in tests with *H. bicinctus* vs. *H. haemorrhoidalis* was the same as for the test with *S. aotearoana* vs. *H. haemorrhoidalis,* except that the *C. robusta* seedlings (the host plant for *S. aotearoana*) were replaced with black nightshade (*Solanum nigrum* L.) seedlings (the host plant for *H. bicinctus*). Damp vermiculite was provided for pupation of *H. bicinctus.* Also, five extra replications were added in the *H. bicinctus* vs. *H. haemorrhoidalis* choice tests due to low levels of parasitism for both species in two of the initial five replicates.

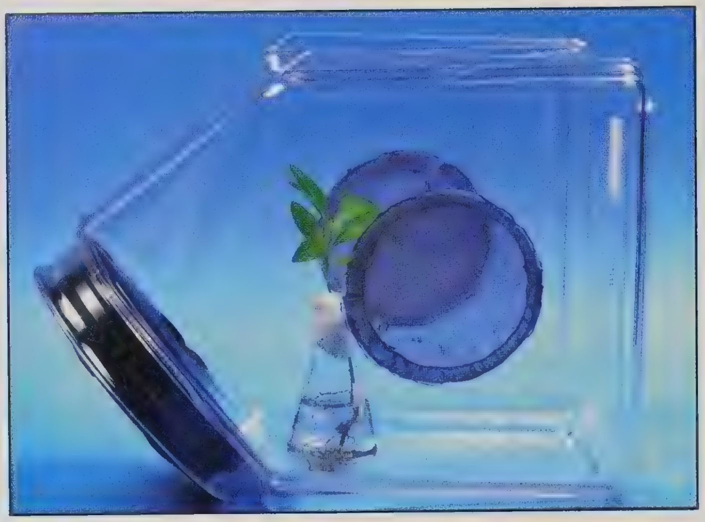

Figure 3. *Coprosma robusta* seedling in a no-choice test container. Photo: M. Henderson.

All experiments were assessed after 14-18 days. The numbers of adult thrips of each species and number of *T. semiluteus* pupae (recognised by their black coloration compared to the cream coloration of healthy thrips pupae) were recorded. Numbers of emerged adult parasitoids were recorded after 20-25 days. Statistical analyses for significant differences were performed using t-tests. Data were analysed using the SAS® statistical package. Percentages were subjected to angular transformation before analysis, but untransformed data are presented.

Results for Test #1 and analysis of problems In the no-choice tests with *S. aotearoana* and *H. haemorrhoidalis*, 92% (avg. 46/50) of the *H. haemorrhoidalis* larvae exposed to *T. semiluteus* were parasitized. By comparison, only 6% (avg. 3/50) *S. aotearoana* larvae were parasitized (Table 1). The percentage of parasitoids completing development and emerging as adults from *H. haemorrhoidalis* was very high (86-95%) and significantly higher ($P < 0.05$) than from *S. aotearoana* (37-53%) (Table 1). *Thripobius semiluteus* parasitized significantly more ($P < 0.05$) *H. haemorrhoidalis* than *S. aotearoana* under both choice and non-choice designs, and significantly more parasitoids completed development on *H. haemorrhoidalis* compared to *S. aotearoana* in each case (Table 1).

In the no-choice tests with *H. bicinctus* and *H. haemorrhoidalis*, there was no significant difference ($P > 0.05$) between the numbers of *H. bicinctus* and *H. haemorrhoidalis* parasitized (Table 1). The percentage of parasitoids completing development and emerging as adults from both hosts was very high and not significantly different ($P > 0.05$) (Table 1). When *T. semiluteus* was provided with a choice, significantly more ($P < 0.05$) *H. haemorrhoidalis* than *H. bicinctus* were parasitized. However, there was no significant difference ($P < 0.05$) between the percentages of resultant parasitoids completing development on *H. haemorrhoidalis* versus *H. bicinctus* (Table 1).

In both choice and no-choice tests, the non-parasitized larval and pupal mortality of *S. aotearoana* was very high at 76.9% (± 13.64 SE) and 98.6% (± 0.9 SE), respectively. As the larvae did not die in the first five days after exposure to *T. semiluteus* (most were at the prepupal

Table 1. Number (of 50) of *H. haemorrhoidalis, S. aotearoana,* and *H. bicinctus* larvae parasitized (mean ± SE) in no-choice and choice tests and percent emergence of adult parasitoids in each species.

Test	Thrips species	Number parasitoid pupae formed	% Emergence of adult parasitoids
No-choice	*H. haemorrhoidalis*	46.4 1.17 a[1]	95.29 1.38 a
	S. aotearoana	3.4 1.12 b	52.68 12.72 b
Choice	*H. haemorrhoidalis*	45.2 2.48 a	93.50 1.45 a
	S. aotearoana	4.4 2.32 b	36.79 12.10 b
No-choice	*H. haemorrhoidalis*	23.8 6.86a	89.03 3.13a
	H. bicinctus	16.8 2.94a	82.65 3.93a
Choice	*H. haemorrhoidalis*	23.1 6.11a	86.02 4.99a
	H. bicinctus	2.9 1.94b	44.67 21.49a

[1] Numbers from each test, within a column, with the same letter are not significantly different (T-test, $P < 0.05$)

stage or late second instars), it was assumed that the mortality was not caused by *T. semiluteus* host feeding on *S. aotearoana*. However, the cause of pupal mortality was further investigated (see Test # 2). Parasitism of *H. haemorrhoidalis* was higher in the earlier tests with *S. aotearoana* (45.2 and 46.4%) than in the *H. bicinctus* tests (23.1 and 23.8%). This may have been due to a drop in the fitness of the parasitoids over a prolonged period (14 months) of laboratory rearing.

TEST #2: SURVIVAL OF *SIGMOTHRIPS AOTEAROANA* UNDER TEST CONDITIONS

Methods for Test #2 To investigate if the presence of *T. semiluteus* caused the high level of *S. aotearoana* mortality seen during the host testing experiments, first instar *S. aotearoana* were held under the same conditions used in Test #1 (see above), except for the absence of *T. semiluteus.* Five replicates of 50 *S. aotearoana* larvae were placed on *C. robusta* seedlings in test arenas and held in the quarantine room for larvae to complete development. After two weeks, damp vermiculite was placed in cages with *S. aotearoana* for pupation. Once the late second instars started showing signs of pupating (stopping feeding and migrating down the seedling), the container was checked every 2-3 days for adults.

Results of Test #2 No *S. aotearoana* reached the adult stage. However, the larvae survived until the late second instar, when they migrated down the seedlings and attempted to pupate. Most *S. aotearoana* died as pre-pupae or pupae. A small number of larvae died in the first two days. This early larval mortality was most likely caused by handling. The failure of *S. aotearoana* to survive in the laboratory made it difficult to assess the potential impact of *T. semiluteus.* All 250 *S. aotearoana* larvae in Test #2 died before becoming adults. This happened in the absence of parasitoids and presumably was caused by physical conditions that were unfavourable for pupation.

TEST #3: POPULATION DYNAMICS OF *SIGMOTHRIPS AOTEAROANA*

Methods for Test #3 We made observations on the life history and field population dynamics of *S. aotearoana* over a 12-month period to detect unrecognized vulnerability to attack by *T. semiluteus.* A population of *S. aotearoana* was sampled in a four-hectare area of native forest in Auckland (41° S, 175° E). Five leaves on each of twenty randomly selected *G. ligustrifolium* seedlings were checked in situ for the presence of first or second instar larvae, pupae, and adults every two weeks.

Results of Test #3 No pupae were found on seedlings, suggesting that pupation occurred in the leaf litter. Adults were present on leaves throughout the year, but larvae were not present in winter, from early June to late October (1996). The population had a patchy distribution and thrips density was low, with a maximum of 1.0 thrips per leaf.

DISCUSSION

INTERPRETATIONS OF TEST RESULTS

Host testing showed that the endemic New Zealand thrips *S. aotearoana* is a potential target for parasitism by *T. semiluteus,* although the target pest, *H. haemorrhoidalis,* is clearly preferred. The low rate of *S. aotearoana* parasitism observed in our tests was not affected by the presence or absence of the target pest, *H. haemorrhoidalis. Thripobius semiluteus* also showed a preference for *H. haemorrhoidalis* over *H. bicinctus* in our choice tests. However, when the parasitoid was provided with *H. bicinctus* alone, the percent parasitism was equivalent to that when *H. haemorrhoidalis* alone was provided. Despite the apparent acceptability of *H. bicinctus* as a host for *T. semiluteus* under laboratory conditions, *H. bicinctus* is not known as a host in the wild in Australia where both species occur together (Froud and Stevens, 1998). Similarly, in the United States, *T. semiluteus* has been reared in *H. femoralis* under laboratory conditions, but it has never been recorded from this host in the field (Loomans and van Lenteren, 1995). These discrepancies between field and laboratory data suggest that laboratory data may not accurately reflect likely host/parasitoid interactions in the natural environment, and, in the case of *T. semiluteus,* our laboratory experiments appear to have overestimated the parasitoid's host range. Goldson *et al.* (1992) stated that the use of choice and no-choice tests in small cages can overestimate the potential field host range of a parasitoid. We suspect that confinement and/or poor host recognition by inexperienced females played an important role in the non-target parasitism seen in our tests.

Given the relatively high level of parasitism (6-34%) of *H. bicinctus* in our tests, the lack of such parasitism in the wild, and the relatively low level of parasitism (7-9%) of *S. aotearoana,* we concluded it is unlikely that *S. aotearoana* would be parasitized in the field. Unlike *H. haemorrhoidalis* and *H. bicinctus* populations in Australia, which are sympatric, *H. haemorrhoidalis* and *S. aotearoana* in New Zealand have very distinct habitats. The pest thrips rarely occurs in the native forest where *S. aotearoana* is found. This habitat separation between the two thrips species should decrease the chances of exposure of *S. aotearoana* to *T. semiluteus.*

Differences between the ecology and biology of *H. haemorrhoidalis* and *S. aotearoana* further decrease the likelihood of *T. semiluteus* establishing permanent populations on *S. aotearoana.* Whereas all life stages of *H. haemorrhoidalis* are present year round, *S. aotearoana* appears to overwinter only as feeding adults or possibly pupae in the soil. Reproductive diapause of *S. aotearoana* would reduce the ability of *T. semiluteus* to successfully establish in areas inhabited only by *S. aotearoana* as no host larvae would be available for parasitism for 4 to 6 months of each year. This implies *T. semiluteus* would need to re-colonize such habitats every spring, following the appearance of the native thrips larvae. *Thripobius semiluteus* is active year round in both California and Australia (Beattie and Jiang, 1990; McMurtry *et al.*, 1991). *Heliothrips haemorrhoidalis* occurs in large colonies with insects in overlapping life stages year round, on a wide range of host plants. Froud (1997) found up to 13 *H. haemorrhoidalis* thrips per leaf on the plant *Acmena smithii* Poiret, compared to one *S. aotearoana* thrips per leaf on *G. ligustrifolium* during the same sampling period. *Thripobius semiluteus* thus has access to large mixed-age colonies of *H. haemorrhoidalis,* which enables the parasitoid to oviposit in many larvae during its short lifespan. The low density and patchy distribution of *S. aotearoana* larvae, combined with this species' habitat separation from *H. haemorrhoidalis,* would further reduce vulnerability of *S. aotearoana* to attack by *T. semiluteus,* should any non-target parasitism occur.

SUMMARY EVALUATION OF THE ASSESSMENT

Completeness Our primary concern was to evaluate the potential for deleterious effects by *T. semiluteus* on indigenous thrips before introducing it to New Zealand. The level of host testing conducted was comprehensive given the parasitoid's very narrow recorded host range. A full Importation Impact Assessment (IIA) report was required as part of the application to import *T. semiluteus* into New Zealand. This report detailed our host-range studies and also discussed several developmental biology aspects of the parasitoid and its host that provided substantial evidence that the potential risk of *T. semiluteus* to indigenous species was negligible. The court hearing and public submission process provided a platform to present our scientific evidence with a high level of transparency.

One remaining concern is that the *T. semiluteus* colony used for New Zealand releases was from Italy (taken from Israel, previously taken from the United States) and potentially might not be the same 'biotype' as the one we tested. An attempt was made to obtain *T. semiluteus* directly from California, but *H. haemorrhoidalis* has become so rare in the field there that all local insectaries have ceased production of *T. semiluteus.* Several recent studies in New Zealand with other groups of parasitoids have shown large host-specificity differences, depending on the biotype introduced (Barratt *et al.*, 1997; Phillips *et al.*, 2002).

Ideally, all three test species should have been tested concurrently rather than 14 months apart because the fitness of *T. semiluteus* apparently declined by the time of the later experiments. Testing all species at once was not possible because of time constraints and the difficulty of rearing enough *S. aotearoana.* Host testing of *T. semiluteus* against *S. staphylinus* (gorse thrips) may also have been justified, given the value of this species in a weed biological control program. However, conceding that this species should be tested, despite extensive evidence that it would not be a host, would have resulted in a requirement that *T. semiluteus* be tested against all 17 species of indigenous thrips species in the subfamily Thripinae. If this had been required, it is unlikely that the *T. semiluteus* biological control introduction program could have been undertaken.

Post-release evaluations *Thripobius semiluteus* was released into New Zealand in February of 2001 at 14 pesticide-free or organic citrus and avocado orchards and several home gardens. Monitoring in late summer of 2002 and 2003 at release sites showed that *T. semiluteus* is locally established at several sites. However, so far there has been very little spread of *T. semiluteus*, which generally has only been found in release sites and directly adjacent orchards. Due to this low rate of spread, it may be 6-10 years before any meaningful monitoring for parasitism of non-target species in natural situations can be undertaken. A research program to study effects on non-target species in a manipulated situation will, however, begin in 2004.

RECOMMENDATIONS FOR FUTURE WORK

Two main recommendations can be given from our tests; the first is that a control group of hosts (ones that are not exposed to the parasitoid) be included to detect any mortality associated with parasitoids, such as host-feeding. In addition, we suspect that in our system confinement contributed to non-target parasitism; therefore, it is recommended that larger cages be used for host range testing and that ventilation be increased to prevent mixing of plant or host volatiles from target and nontarget species. Also, field records of a lack of parasitism of one of the test species (*H. bicinctus* in Australia) were crucial in suggesting that the low level of attack on this species in our small-cage laboratory tests (and by extension similar attack on *S. aotearoana*) was most likely an artefact.

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CHAPTER 9. *MICROCTONUS* PARASITOIDS AND NEW ZEALAND WEEVILS: COMPARING LABORATORY ESTIMATES OF HOST RANGES TO REALIZED HOST RANGES

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FORAGE WEEVIL PESTS IN NEW ZEALAND AND THEIR PARASITOIDS

The pest weevils *Sitona discoideus* Gyllenhal and *Listronotus bonariensis* (Kuschel) have been the targets of recent biological control programs in New Zealand forage crops. Two species of *Microctonus* parasitoids (Hymen.: Braconidae: Euphorinae) were introduced to control these pests. For these parasitoids, retrospective laboratory host range testing and field studies were carried out in order to test the degree to which laboratory data can predict field host ranges.

THE PEST WEEVILS

***Sitona discoideus* in alfalfa** Alfalfa is an important forage crop in low rainfall areas of New Zealand, particularly the eastern and central rain shadow areas of the South Island and parts of the North Island. Alfalfa out-produces grass pasture in the 300-800 mm rainfall zone (Douglas *et al.*, 1987). However, in the last 20 years, alfalfa production has declined, partly because of increased pressure from exotic pests (Douglas *et al.*, 1987). *Sitona discoideus* was first discovered in New Zealand in 1974 (Esson, 1975). Originating from the Mediterranean region, *S. discoideus* feeds on legumes in the genus *Medicago*. Adults feed on foliage, but the larvae are the most damaging stage, feeding within the root nodules of plants and reducing the ability of the plant to fix nitrogen. High densities of newly emerged adult *S. discoideus* can severely defoliate an alfalfa crop (Goldson *et al.*, 1984).

***Listronotus bonariensis* in pasture** *Listronotus bonariensis* (Argentine stem weevil) was first recorded in New Zealand in 1927 (Marshall, 1937). The larva is a stem borer that feeds within the tillers of ryegrass and other grass species, and the adults feed on foliage. It has become one of New Zealand's most widespread and serious pests, thought to cause pasture production losses of up to NZ$250 million per year. Losses occur as a result of reduced plant production, but also because the pest causes a change in pasture quality that can cause health problems in livestock (Prestidge *et al.*, 1991).

BIOLOGICAL CONTROL AGENTS

Microctonus aethiopoides For biological control of *S. discoideus*, the braconid parasitoid *Microctonus aethiopoides* Loan (Figure 1) was released in New Zealand in 1982 from Australia (Stufkens *et al.*, 1987), where it had been introduced earlier from the Mediterranean region (Cullen and Hopkins, 1982; Aeschlimann, 1983). Although ecotypes from Greece and France were introduced to Australia along with those from Morocco, it is thought that the Moroccan strain is the one that was released in New Zealand. Surveys throughout New Zealand have shown that *M. aethiopoides* is well established in *S. discoideus* populations in alfalfa-growing areas (Stufkens *et al.*, 1987; Ferguson *et al.*, 1994), where it has been shown to suppress *S. discoideus* populations (Goldson *et al.*, 1993). *Microctonus aethiopoides* was, however, released with limited host range testing in quarantine, which revealed no evidence of attack on non-target species (M. Stufkens, pers. comm.). In its natural range, *M. aethiopoides* is known to parasitize weevils in the genera *Hypera* (3 spp.) and *Sitona* (about 8 spp.) (Loan, 1975; Aeschlimann, 1980). A survey of alfalfa in southeastern Australia undertaken in November 2001 found evidence of non-target parasitism in only one Australian native species, an undescribed species of Prosayleus (Barratt *et al.*, in press).

Microctonus hyperodae This parasitoid (Figure 2) is of South American origin and was released in 1991 at several sites throughout New Zealand for control of *L. bonariensis*. This parasitoid has established successfully (Goldson *et al.*, 1994ab), although spread from the more southerly release sites has been slow (Ferguson *et al.*, 1997). *Microctonus hyperodae* was released after extensive quarantine testing (Goldson *et al.*, 1992). These tests suggested that the parasitoid was oligophagous, and the authors predicted that one or two native weevil species might be parasitized in the field. Collections of the parasitoid were made from seven South American locations from ecologically different environments in Argentina, Brazil, Uruguay, and Chile. These ecotypes were maintained separately in the laboratory rearing process, and for each major release, equal numbers of individuals of each of the ecotypes were released at each site so

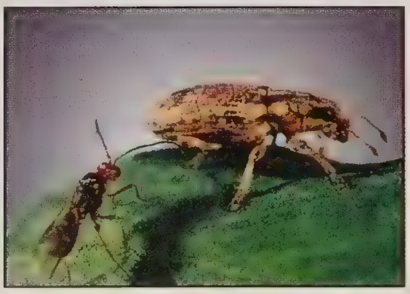

Figure 1. *Microctonus aethiopoides* with *Sitona discoideus*. Photo: Mark McNeill. (UGA1295011)

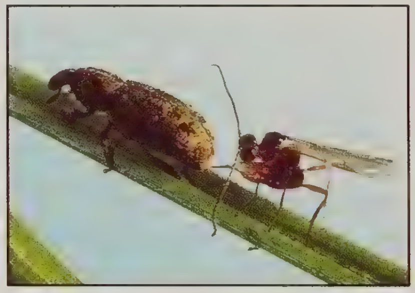

Figure 2. *Microctonus hyperodae* with *Listronotus bonariensis*. Photo: Mark McNeill. (UGA1295012)

that information on differences in suitability of the different ecotypes could be determined. After three years it became apparent that the east coast ecotypes (Argentina, Brazil, Uruguay) had established more successfully than those from the west coast (Chile) (Goldson *et al.*, 1997).

Very little is known about the natural host range of *M. hyperodae*. Loan and Lloyd (1974) found that in the field in western Patagonia, *M. hyperodae* attacked only *L. bonariensis*, although other species in the genus were present. This apparent monophagy provided further evidence of the suitability of the parasitoid for biological control of *L. bonariensis* in New Zealand.

IDENTIFYING POTENTIAL NON-TARGET HOSTS IN NEW ZEALAND

AFFINITIES BETWEEN THE TARGET PESTS AND THE NEW ZEALAND FAUNA

Likely potential non-target hosts of *M. aethiopoides* and *M. hyperodae* were native New Zealand weevils found near agricultural areas where the biological control agents were released. The target hosts for *M. aethiopoides* (*S. discoideus*) and *M. hyperodae* (*L. bonariensis*) are in the subfamily Entiminae, tribes Sitonini and Rhytirhinini, respectively, using the classification scheme of Leschen *et al.* (2003). Alonso-Zarazaga and Lyal (1999) considered the Sitonini and Tropiphorini to be so closely related that they should perhaps be combined. Tropiphorini and Rhytirhinini are well represented in New Zealand by native species, especially the former, many of which inhabit pastures and natural grasslands (Table 1). The native weevils of New Zealand are not well known taxonomically, and many species in these tribes are undescribed, with some probably still undiscovered. Knowledge of the ecology and biology of these native weevils is limited, which makes it difficult to know if the phenology of susceptible stages of the native species resembles that of the introduced pest weevils. Conversely, if native weevils are present at times when the target hosts are scarce, such timing might place the native species at increased risk. Information for some species has been gathered (Barratt *et al.*, 2000).

Table 1. Numbers of native weevils potentially at risk from *Microctonus* spp. introductions in New Zealand.

	Number of native weevil species with given degree of relatedness to introduced weevil (*Sd* or *Lb*) or number in group of special concern	
	S. discoideus	*L. bonariensis*
In same genus	0	0
In same tribe	23[1]	4
In same subfamily (Entiminae)	31	31
Valued biological control agents	2	2
Species of conservation concern	19	19

[1] includes Sitonini + Tropiphorini

To determine which New Zealand weevils might be at risk from these introduced biological control agents, a survey of over 150 pastures and alfalfa fields was carried out as part of a retrospective case study. Eighty-five species of Curculionoidea were collected, of which 75% were native (Barratt *et al.*, 1998). Thirty-two species were in the Tropiphorini, of which 84% were in the endemic genera *Irenimus* and *Nicaeana.* At many sites, species of native weevils (especially entimines) and the non-native pests were found in mixed populations at similar population densities (Barratt *et al.*, 1998). Furthermore, both of the exotic pest weevils were frequently found up to sub-alpine elevations in native vegetation (Dickinson *et al.*, 1998). Consequently, many additional native weevil species in a wide range of agricultural and natural grassland environments could potentially come into contact with the biological control agents. In addition, three native species of *Microctonus* parasitoids were discovered in New Zealand (Shaw, 1993), which are potentially at risk of being displaced by the introduced parasitoids. For only one of these species, *Microctonus zealandicus* Shaw (a gregarious parasitoid), is the host known: the native entimine *Irenimus aequalis* Broun.

AT RISK SPECIES OF SPECIAL VALUE

Two weevils, *Rhinocyllus conicus* Froelich and *Trichosirocalus horridus* (Panzer), have been introduced into New Zealand to control the target weed nodding thistle (*Carduus nutans* L.) in pasture and alfalfa. These two weed biological agents are likely to come into contact in the field with the parasitoids *M. aethiopoides* and *M. hyperodae* and might be parasitized.

The New Zealand Department of Conservation has in recent years recognized invertebrates as an important and dominant component of New Zealand's indigenous biodiversity. Among the invertebrates listed in recent reports as either 'nationally critical' (with a high risk of extinction) or 'nationally endangered' are four weevils (Hitchmough, 2002). Fifteen other weevils are considered 'nationally threatened', requiring conservation action (McGuinness, 2001).

DEVELOPMENT OF SPECIES LIST FOR HOST RANGE TESTING

Host range testing for *M. aethiopoides* was carried out in the early 1980s, when regulatory requirements for demonstrating environmental safety were less rigorous, and the methodologies used were not published or well documented. For *M. hyperodae*, host range testing was much more thorough and the results were well documented (Goldson *et al.*, 1990). These prerelease laboratory tests showed that four out of 23 test species were parasitized by *M. hyperodae*, and in all but one species, parasitoid development was unsuccessful or retarded (Goldson *et al.*, 1992). For both biological control agents, further testing was conducted as part of a retrospective study to determine the extent to which laboratory host range testing might have predicted the field host range (Barratt *et al.*, 1997). The rationale for selecting a list of non-target species for testing is shown in Table 2, which also lists some species tested subsequently.

Since our knowledge of the native weevil fauna is far from complete in New Zealand, it is not possible to calculate the proportion of the species of Entiminae that were represented in laboratory tests. Only four of a total of 31 genera found on the two main islands were included in tests, but the proportion of species tested would be less than 5%.

Table 2: Species selected (✓) for retrospective host range testing with *Microctonus aethiopoides* (*Ma*) or *Microctonus hyperodae* (*Mh*) and rationale for choosing each species.

Species	*Ma*	*Mh*	Rationale
I. Same as Target at Order Level[a]			
Allocharis sp. (Chrysomelidae)	✓		
II. Same as Target at Family Level[b]			
Peristoreus cruciger (Broun)	✓		Native Curculioninae, common in native grasslands
Rhinoncus australis Oke	✓	✓	Introduced Curculioninae, common in North Island pastures
III. Same as Target at Subfamily Level[c]			
Phlyctinus callosus Boheman	✓		Introduced pest species, same subfamily as target, Entiminae
IV. For *Ma* same as Target at Tribe Level[d]			
Irenimus aemulator (Broun)	✓	✓	
Irenimus aequalis (Broun)	✓	✓	
Irenimus egens (Broun)	✓	✓	
Irenimus stolidus Broun	✓	✓	
Irenimus similis (Barratt & Kuschel)	✓		
Nicaeana cervina Broun	✓	✓	
Nonnotus albicans Broun	✓		
Protolobus porculus (Pascoe)	✓		Very limited distribution in native and agricultural grassland
Zenagraphus metallescens Broun	✓		Native, common in South Island sub-alpine grasslands
V. For *Mh* same as Target at Tribe Level[d]			
Steriphus delaiguei (Germain)	✓	✓	Introduced; common in agricultural grassland
Steriphus variabilis Broun	✓		Native; common in native and agricultural grassland
VI. Same as Target at Genus Level[e]			
Sitona lepidus Gyllenhal	✓	✓	Introduced pest

Table 2: Species selected (✓) for retrospective host range testing with *Microctonus aethiopoides* (*Ma*) or *Microctonus hyperodae* (*Mh*) and rationale for choosing each species (continued).

Species	*Ma*	*Mh*	Rationale
VII. Species in same genus as endangered species			
Anagotus latirostris (Broun)		✓	Native, limited distribution in Central Otago alpine herbfield
VIII. Biological control agents			
Rhinocyllus conicus Froelich	✓	✓	Introduced weed biological control agent
Trichosirocalus horridus (Panzer)	✓	✓	Introduced weed biological control agent

[a] Beetles other than weevils (Curculionidae); species found in native grassland that have similar behavioral and habitat characteristics and similar size to one of the target hosts.

[b] In the Curculionidae, but in subfamilies different from that of the target pests, i.e., not in Entiminae

[c] In the Entiminae, but not in either of the tribes Triopiphorini or Rhytirhinini

[d] For M. aethiopoides this is other species of Tropiphorini; for M. hyperodae, this is Rhytirhinini; this group is composed of species considered at risk because they inhabit native or agricultural grassland

[e] Ether another species of Sitona or Listronotus

LABORATORY HOST RANGE TESTS

Rearing Conditions

Tests were conducted in an insect rearing room maintained at 20 ± 2°C with a relative humidity of 40-60%, and a photoperiod of 16:8 (L:D) hours. This is within the range of 'average' field conditions that would be expected during the day in summer in New Zealand. Weevils were contained in plastic cages (160 by 180 mm by 75 mm deep) with a fine-gauze lid (Figure 3). The floor of the cage was fitted with plastic mesh with holes 1 by 1 mm, and this cage was inserted

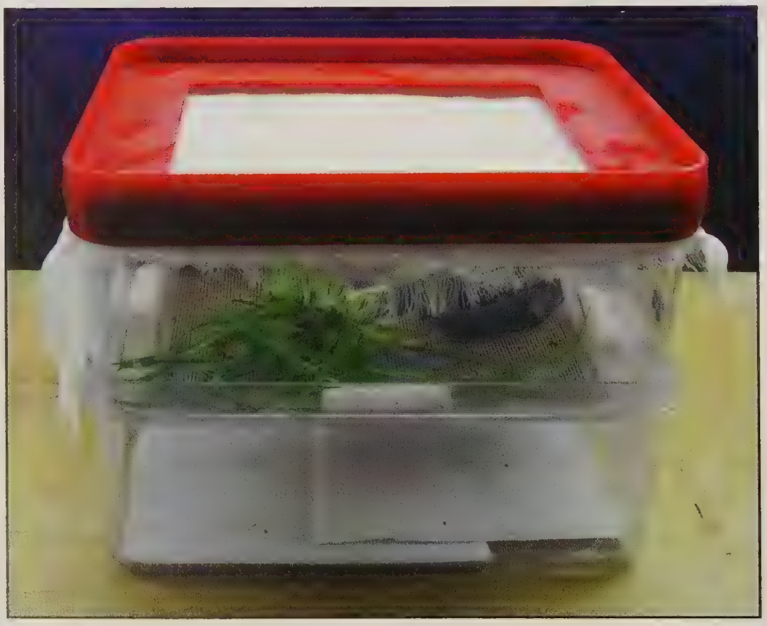

Figure 3. Cage used for standard host range tests. Photo: Barbara Barratt.

into the top of another similar container with textured absorbent paper towel covering the base. The paper served as a substrate for pupation of emergent prepupal parasitoids, which moved down through the mesh from the upper to the lower cage. Cages were selected on the basis of size. They needed to be large enough to spaciously accommodate about 20 weevils and two bundles of alfalfa, yet small enough to be handled easily, and housed in a controlled temperature rearing laboratory in large numbers to allow for experiments with adequate replication. In general, the aim of laboratory tests is to replicate 'natural' field conditions as closely as possible, but this is invariably a compromise. Host densities are likely to be much higher in cages than in the field (in our cages, weevil densities would be close to 700 per m^2, which could occur in the field at the upper range of density), and the environment within a cage is likely to be far less complex than the field environment. McNeill (2000) found that parasitoid activity and weevil parasitism was significantly higher in Petri dishes compared with laboratory cages, concluding that in a smaller space, the number of encounters between host and parasitoid increased. Evans *et al.* (1997) found that parasitism of native weevils by *M. aethiopoides* was 40-55% in laboratory cages as described above but averaged only 15% in large field cages (45 x 90 x 50 cm high). The level of parasitism obtained in the field cages was similar to that recorded in an open field population nearby. Proportions of failed parasitism and superparasitism in the field cages were similar to that in laboratory cages.

INSECT FEEDING

Listronotus bonariensis and *S. discoideus* were provided with Grasslands cv. Manawa ryegrass ([*Lolium perenne* x *L. multiflorum*] x *L. perenne*) and Grasslands cv. Wairau alfalfa, *Medicago sativa* L., respectively. Native weevils survived best in the laboratory when given both ryegrass and alfalfa, and pollen grains (Grainger, 1995). *Rhinocyllus conicus* was provided with foliage of nodding thistle. Ryegrass and alfalfa plants were grown in commercial seed-raising mix in glasshouse trays with cells 2.7 by 2.7 cm, 4.5 cm deep, sown with 6-10 seeds per cell. The plants were grown to »10 cm high, removed intact, and the roots and soil enclosed in a plastic bag (10 by 7.5 cm) secured firmly at the base of the plants using a plastic cable clip to prevent weevils entering the bags. One or two bags of plants were placed in each cage and replaced with fresh plants every 3 to 4 days. Water was supplied using saturated cotton dental wicks placed in the cages, which were resoaked every 1 to 2 days and at each change of food.

TEST SPECIES AND PARASITOID COLLECTION

Weevils were collected from the field, in most cases by using a commercial leaf-sucking machine (Blower Vac) fitted with a gauze collection bag immediately behind the inlet of the machine or by sweep-netting (Barratt *et al.*, 1997). The thistle biological control agents *R. conicus* and *T. horridus* were collected directly from thistle plants in the field, and the native weevils *A. latirostris* and *Zenagraphus metallescens* Broud were collected by hand from native vegetation.

Microctonus aethiopoides was reared from *S. discoideus* collected from the field, and *M. hyperodae* was reared from *L. bonariensis* from a laboratory colony at AgResearch, in Lincoln, New Zealand. Newly emerged parasitoids were provided with a water-honey solution and held for up to four days before being used in an experiment. *Microctonus aethiopoides* females were confined with males to allow mating to occur before being used in tests. This was not necessary for *M. hyperodae*, which is parthenogenetic.

HOST RANGE TEST PROCEDURE

Using a no-choice design, a standard host/parasitoid ratio and procedure was followed as described in Barratt *et al.* (1996). Twenty weevils were placed in each cage (Figure 3) and exposed to three female parasitoids for 48 hours. Depending upon the availability of weevils, five replicate cages of the non-target test species were exposed and five cages were unexposed. Where possible, five exposed and five unexposed replicate cages of the target host were run in parallel with one or more of the test species to provide a positive control. After the 48-hour exposure period, parasitoids were removed and the weevils maintained until the resulting parasitoid prepupae emerged.

Emergent parasitoid pupae were recorded daily and removed from the cages to Petri dishes containing a water-soaked dental wick to maintain high humidity. Newly eclosed adults also were recorded daily, allowing comparison of parasitoid developmental periods between rearing from test and target species.

Each experiment was terminated when no further parasitoid prepupae emerged from weevils for at least two days, or after 30 days if no prepupae had emerged. Surviving weevils, as well as those that died during the experiment, were dissected (Figure 4). The presence of parasitoid larvae in these weevils was recorded and such hosts added to those which parasitoids had emerged to give total percentage parasitism. Any signs of a host immune response, including melanization of parasitoid eggs or larvae, encapsulation, or malformed, emaciated larvae were noted, as well as incidence of super-parasitism.

The testing procedure described above was standardized so that results would be comparable between tests, and a no-choice rather than choice design was used so that the maximum physiological host range could be observed. We were interested in determining the full range of

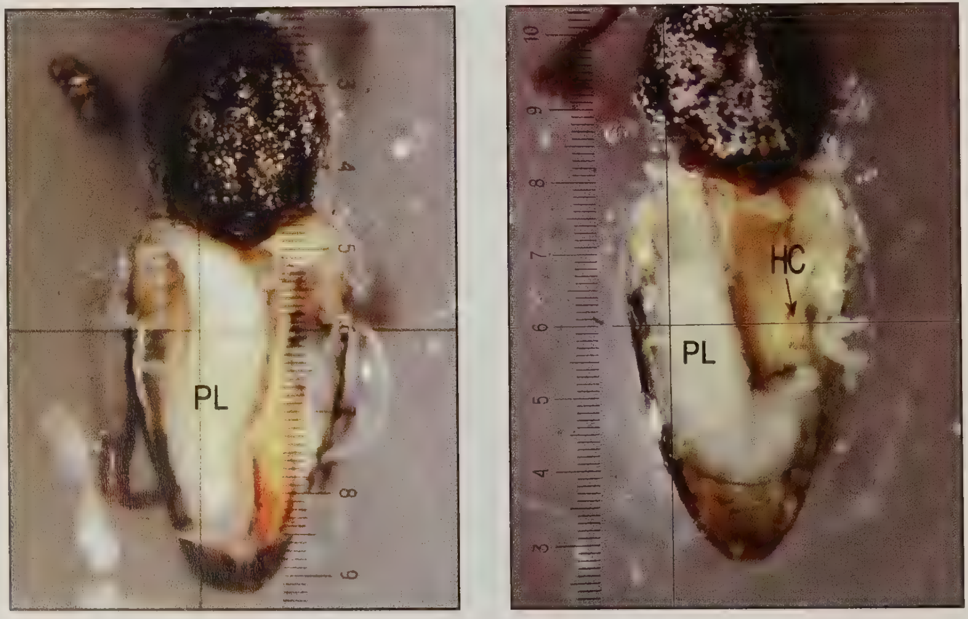

Figure 4. New Zealand native weevils dissected to show *M. aethiopoides* parasitoid larvae (PL) and 1st instar head capsule (HC). Photos: Barabara Barratt. (UGA1295013)

potential hosts rather than comparing host preferences. In the field, a choice of hosts may not always be available to parasitoids, especially if they emerge from hosts that have carried them away from the target host's environment. This seems to occur quite commonly when parasitized *S. discoideus* disperse after aestivation in summer. It is also quite common to find low numbers of *L. bonariensis* in native grassland, and parasitized *L. bonariensis* have been encountered in sub-alpine habitat. In the absence of the target hosts, as occurs when *S. discoideus* is in summer aestivation, parasitoids may be forced to seek suitable alternative hosts among native species.

INTERPRETATIONS OF TEST RESULTS

Laboratory Test Results

Microctonus aethiopoides This species was been found to oviposit in 9 of 11 species of native Curculionidae (14 of 19 total Curculionidae species) to which it was exposed in the laboratory (Table 3), with levels of parasitism often equivalent and sometimes higher than those achieved in the target host, *S. discoideus,* in parallel tests. Immature parasitoid development times in native weevils (from when adult parasitoids were removed from test cages to when parasitoid prepupae emerged from the test species) were similar to that in *S. discoideus.* Of parasitoids that emerged as pre-pupae from native species, about 80% developed to the adult stage, but 34% of the parasitoids found during dissection (of hosts from which no parasitoids emerged) were melanized or showed other signs of a host immune response. Most of these cases occurred in the test species that were most distantly related to the target host. No melanized larvae were found in dissections of *S. discoideus.*

In a later laboratory study, Barratt and Johnstone (2001) found that superparasitism occurred in the native host *Nicaeana cervina* Broun more frequently than would be expected. We also found that successful development of *M. aethiopoides* larvae in *N. cervina* was more likely to occur if the host had been superparasitized, suggesting that multiple parasitism helps suppress host defences in this novel host. A virus-like particle (MaVLP), structurally similar to polydnavirus, has been found in the ovaries of female *M. aethiopoides* (Barratt *et al.*, 1999), and this or other parasitoid-derived secretions may be transmitted to hosts during parasitoid oviposition, as is the case in other braconids and ichneumonids (Beckage, 1998).

Both of the two weed biological control agents tested, *R. conicus* and *T. horridus,* were parasitized in laboratory tests, although in the case of *T. horridus,* this was recorded only once and the parasitoid larvae did not develop successfully. About 40% of *R. conicus* exposed to *M. aethiopoides* were parasitized successfully, and parasitism of this host has also been recorded in the field (Table 3). Although *R. conicus* was exposed to *M. aethiopoides* in pre-release tests, no parasitism was recorded at that time; this may have been because the tests were undertaken in autumn when *R. conicus* was probably in diapause and inactive. It is known that *M. aethiopoides* requires an active host to stimulate stalking behaviour and oviposition (Loan and Holdaway, 1961).

Table 3. Results of laboratory testing (L) with *M. aethiopoides* (*Ma*) and *M. hyperodae* (*Mh*) and parasitism recorded in the field (F) either by rearing or dissection.

Test species	***Ma***		***Mh***		**Habitat and Reference**
	L	**F**	**L**	**F**	
Curculionoidea					
Anagotus latirostris (Broun)	-	-	N	-	alpine cushionfield; Goldson *et al.*, 1992 for Mh in lab; Barratt *et al.*, 1997
Atrichonatus taeniatulus (Berg)*	-	Y	-	N	alfalfa; Barratt *et al.*, 1997
Brachyolus obscurus Sharp	-	N	-	N	pasture
Bryocatus spp.	-	N	-	N	native grassland and developed pasture
Catoptes censorius Pascoe	-	N	-	N	pasture
Catoptes cuspidatus (Broun)	-	N	N	N	native grassland/shrubland; Goldson *et al.*, 1992 for *Mh* in lab
Catoptes murinus (Broun)	-	N	-	N	native sub-alpine hebfield
Catoptes robustus Sharp	-	N	Y	N	alpine cushionfield; Goldson *et al.*, 1992 for *Mh* in lab
Catoptes sp. cf. *scutellaris* Sharp	-	N	-	N	shrubland
Cryptorhynchinae sp.	-	N	-	N	native grassland
Eugnomus sp.	-	Y	N	N	subalpine herbfield; Goldson *et al.*,1992 for *Mh* in lab
Desiantha sp.	-	-	N	-	pasture; Goldson *et al.*, 1992 for *Mh* in lab
Epitemetes grisealis Broun	-	N	-	N	Pasture
Epitimetes sp.1	-	N	-	N	Pasture
Epitimetes sp.2	-	N	-	N	Pasture
Exapion ulicis (F.)	-	N	N	N	introduced gorse biocontrol agent; Goldson et al., 1992 for Mh in lab
Gromilus sp.	-	-	N	-	forest; Goldson *et al.*, 1992 for *Mh* in lab
Hoplocneme cyanea	-	-	N	-	forest; Goldson *et al.*, 1992 for *Mh* in lab

Y = parasitism recorded in test or sample; N = parasitism not recorded; - = species not tested; * = introduced species.

Table 3. Results of laboratory testing (L) with *M. aethiopoides* (*Ma*) and *M. hyperodae* (*Mh*) and parasitism recorded in the field (F) either by rearing or dissection (continued).

Test species	***Ma***		***Mh***		**Habitat and Reference**
	L	**F**	**L**	**F**	
Curculionoidea (continued)					
Irenimus aemulator (Broun)	Y	Y	Y	N	native grassland and pasture; Barratt *et al.*, 1997
Irenimus aequalis (Broun)	Y	Y	Y	Y	pasture; Barratt et al.,1997; Goldson *et al.*, 1992 for *Mh* in lab
Irenimus albosparsus (Broun)	-	Y	-	N	pasture; Barratt *et al.*, 1997
Irenimus compressus (Broun)	-	N	N	N	pasture; Goldson *et al.*,1992 for *Mh* in lab
Irenimus egens (Broun)	Y	Y	Y	N	pasture; Barratt *et al.*, 1997
Otiorhynchus sulcatus (F.)	N	N	-	N	garden
Peristoreus cruciger (Broun)	N	N	-	N	olive trees
Peristoreus veronicae (Broun)	-	-	N	-	Goldson *et al.*, 1992 for *Mh* in lab
Peristoreus sp.	-	N	-	N	native grassland/shrubland
Praolepra infusca Broun	-	N	N	N	native shrubland; Goldson *et al.*,1992 for *Mh* in lab
Protolobus porculus Pascoe	Y	N	N	N	pasture; Barratt *et al.*, 1997
Phlyctinus callosus Boheman	N	N	N	N	pasture; Barratt *et al.*,1997
Rhadinosomus acuminatus	-	-	N	-	Goldson *et al.*,1992 for *Mh* in lab
Rhinocyllus conicus (Froelich)*	Y	Y	N	N	introduced thistle biological control agent; Barratt *et al.*, 1997 and Goldson *et al.*,1992 for *Mh* in lab
Rhinoncus australis Oke*	Y	N	N	?	Barratt *et al.*,1997; positive *Mh* in the field unconfirmed
Rhopalomerus sp.	-	-	N	-	forest; Goldson *et al.*, 1992 for *Mh* in lab
Sitona discoideus Gyllenhal	Y	Y	N	N	alfalfa
Sitona lepidus Gyllenhal*	Y	Y	N	Y	pasture; Barratt *et al.*, 1997

Y = parasitism recorded in test or sample; N = parasitism not recorded; - = species not tested; * = introduced species.

Table 3. Results of laboratory testing (L) with *M. aethiopoides* (*Ma*) and *M. hyperodae* (*Mh*) and parasitism recorded in the field (F) either by rearing or dissection (continued).

Test species	***Ma***		***Mh***		**Habitat and Reference**
	L	**F**	**L**	**F**	
Curculionoidea (continued)					
Steriphus ascitus (Pascoe)	-	-	N	-	pasture; Goldson *et al.*,1992 for *Mh* in lab
Steriphus diversipes lineatus (Pascoe)	-	N	N	N	pasture; Goldson *et al.*, 1992 for *Mh* in lab
Steriphus variabilis Broun	Y	Y	N	Y	pasture; Goldson *et al.*, 1992 for *Mh* in lab; Barratt *et al.*, 2000 for *Mh* in field
*Trichosirocalus horridus Panzer**	Y	N	N	N	introduced thistle biocontrol agent; Goldson *et al.*, 1992 for *Mh* in lab; Barratt *et al.*, 1997
Zenagraphus metallescens Broun	Y	N	-	N	alpine herbfield; Barratt *et al.*, 1997
Total non-target positives	14	16	7	3	
Total negatives	5	33	24	45	
Grand total (non-target)	19	49	31	48	
% positives (non-target)	73.7	32.6	22.6	6.3	
Other Coleoptera					
Eucoides suturalis Pascoe (Cerambycidae)		N		N	Pasture
Chaetocnema nitida (Broun) (Chrysomelidae)		N		N	native grassland
Allocharis sp. (Chrysomelidae)	N	N		N	sub-alpine herbfield
Archeocrypticus topali Kaszab (Archeocrypticidae)			N		alfalfa

Y = parasitism recorded in test or sample; N = parasitism not recorded; - = species not tested; * = introduced species.

In tests with *M. aethiopoides*, dissections showed that 2% of the field-collected *I. aemulator* weevils used in tests as controls (not exposed to parasitoids) had already been parasitized in the field by *M. aethiopoides.*

Microctonus hyperodae Combining pre-release studies (Goldson *et al.*, 1992) and retrospective host range tests on *M. hyperodae* (Barratt *et al.*, 1997), 31 weevil species were exposed to this parasitoid, of which 21 were New Zealand native species, and successful oviposition occurred in five species. However, non-target parasitism levels were much lower in comparison with parallel tests with *L. bonariensis.* Furthermore, in the five native species parasitized, parasitoids emerged from only 3% of the weevils exposed. The proportion of parasitoids found during dissection that were melanized or showed other signs of a host immune response was over 40% in native weevils, compared with about 8% in *L. bonariensis.* Unwanted parasitism in field-collected weevils used in tests was low (3% of the unexposed *N. cervina* and 4% of the unexposed *I. aemulator* were parasitized by *M. aethiopoides* from earlier field parasitism).

The "endangered" weevils now listed in New Zealand as of conservation concern are in the genera *Lyperobius, Anagotus, Stephanorhynchus,* and *Hadramphus.* The work reported here predates this designation. One species of *Anagotus* (*A. latirostris*) was included in the list of weevils exposed to *M. hyperodae* both before its release (Goldson *et al.*, 1992) and in retrospective host range tests (Barratt *et al.*, 1997), and in both cases results were negative (Table 3).

FIELD PARASITISM

Eleven New Zealand native species and five introduced species have been found to be parasitized by *M. aethiopoides* in the field (Table 3), mostly in the agricultural environment. However, *L. bonariensis* collected in modified vegetation (a ski field) at 1650 m were found parasitized by *M. aethiopoides,* and a number of other native weevils collected from native grassland at 500 to 1000 m have also been found parasitized by *M. aethiopoides.* Excluding records where the sample size was less than 10, parasitism levels ranged from 1.6 to 71.4%, the highest parasitism being in *I. aemulator* collected from a pasture in Otago. Of the eleven weevil species parasitized by *M. aethiopoides* in the laboratory, three have as yet not been found parasitized in the field – *P. porculus, Z. metallescens,* and *T. horridus.*

Microctonus hyperodae has been recovered from three non-target species in the field: the native species *I. aequalis* and *Steriphus variabilis* Broun, and the recently discovered exotic species *Sitona lepidus* Gyllenhal (Barratt *et al.*, 1996). Both *I. aequalis* and *S. lepidus* host records were from Waikato (North Island of New Zealand), and in both instances, only a single parasitized host was found. A small number of *S. variabilis* have been found parasitized by *M. hyperodae* in the South Island in Canterbury (Barratt *et al.*, 2000) in pasture where parasitism of the target host was moderately high at the time when non-target parasitism occurred.

COMPARISON BETWEEN PREDICTED AND REALIZED FIELD PARASITISM

Predictions about the likely host range of *M. aethiopoides* and *M. hyperodae,* which were made after retrospective laboratory investigations were complete and for *M. hyperodae* before its release, were generally borne out by what was found in field studies. *Microctonus aethiopoides* has proved to be polyphagous, developing successfully with quite high levels of parasitism in a variety of non-target taxa in a range of habitats. *Microctonus hyperodae* has to date proved perhaps even more oligophagous than was anticipated with only three confirmed nontarget species, and indeed very few individuals having been discovered parasitized in the field. Significantly, one of those detected in the field was the native species *I. aequalis,* which was predicted from the quarantine investigation to be a likely host for *M. hyperodae* (Goldson *et al.*, 1992).

In this comparison between the two parasitoid species, allowance must be made for the fact that *M. hyperodae* is currently less widely distributed throughout New Zealand than *M. aethiopoides* and has been present for only 13 years, compared to 22 years for *M. aethiopoides.*

In general, weevils in the Entiminae appear to be more at risk from parasitism by *M. aethiopoides* than more distantly related taxa, as might be expected. This manifests itself in terms of a higher proportion of weevils attacked in the laboratory and more successful parasitoid larval development. However, field studies have shown that coexistence in the same habitat is also an important factor in susceptibility to attack by parasitoids. For example, *R. conicus* (Curculioninae), which is not uncommonly parasitized by *M. aethiopoides* in the field, is more distantly related to *S. discoideus* than, say, the genus *Catoptes* (Entiminae), which has not been recorded in the field as a host for *M. aethiopoides*. However, *R. conicus* is often found on nodding thistle (*C. nutans*) plants growing as weeds in alfalfa, and hence comes into contact with *M. aethiopoides*. In contrast, many *Catoptes* species are found in shrubland, a habitat where *M. aethiopoides* is likely to be less common.

Unfortunately the higher classification of Curculionoidea remains a contentious issue, and so it is not possible to analyse phylogenetic relationships between taxa and determine whether phylogeny and potential host range are closely linked, as tends to be the case for weed biological control agents and their host plants. As indicated above, while 'relatedness' might determine physiological host range at a broad level, ecological affinity and insect behaviour also appear to be important determinants of field non-target parasitism in this system.

PROBLEMS ENCOUNTERED

OBTAINING TEST SPECIES

Collecting sufficient specimens from the field for host range tests was sometimes difficult, especially from natural as opposed to agricultural grassland areas. In some cases, this was a limitation when attempting to design robust, well replicated tests. Rare and endangered species could not be included in tests, even though these are the very species of greatest concern. In these instances, however, other species in the same genus were tested, when possible.

INSECT PHYSIOLOGICAL CONDITION

It is important to ensure that the individuals of a test species used in a host range test are in an appropriate physiological condition when presented to parasitoids. *Microctonus* species require an active host so that the wasps are stimulated to approach and stalk a potential host. If the host is either moribund or in a physiologically quiescent state (e.g., in diapause), the wasp will not attempt to oviposit and hence will give a misleading result. We consider that this may have occurred when host range testing was being carried out with *M. aethiopoides* before its release. At that time, *R. conicus* was included in tests but no parasitism was recorded. Subsequent tests have shown that in fact *M. aethiopoides* does parasitize *R. conicus* in the laboratory and in the field. We believe that the original tests were carried out in autumn when *R. conicus* is normally in diapause and very inactive. It is possible that approval to release *M. aethiopoides* into the field may not have been granted had it been shown that *R. conicus*, a weed biological control agent, might be adversely affected.

The plants present during a test should be standardized so that test and control insects are held in similar circumstances to avoid any differential effects occurring as a result of plant-derived volatiles. For example, if an alfalfa-feeding insect is being exposed to a parasitoid in parallel with the target host which is a grass-feeder, then both the appropriate grass species and alfalfa should be placed in all cages.

SUMMARY

A retrospective study of non-target parasitism by *Microctonus* spp. in New Zealand was carried out with the objective of developing widely applicable, robust, but feasible methods for pre-release evaluation of non-target effects of proposed biological control agents. The research aimed to contribute to improved decision support for the appropriate regulatory agency in New Zealand, the Environmental Risk Management Authority (ERMA New Zealand), and the Department of Conservation. The study compared laboratory tests, which could be carried out in quarantine, to the host ranges as realized in the field for validation.

Since no biological control agent safety testing program can be totally exhaustive, there is a sequence of steps that can be taken to minimize the chances of adverse effects. Largely, these are based upon protocols adopted by weed biological control practitioners and adapted to suit insect parasitoids. Lack of complete taxonomic and ecological information about the insect fauna in most countries (e.g., compared with plants) makes prediction difficult.

The case studies reported here have hopefully provided some useful information that can be adopted for pre-release host range testing of any parasitoid. To summarize, we have found that the following points may be particularly important:

- Understand as fully as possible the phylogeny, ecology, and phenology of the target host(s) of the proposed biological control agent.
- Identify as fully as possible the elements of the fauna in the area of proposed introduction that might be at risk as a result of taxonomic and ecological affinity to the target pest.
- Consider parasitoid ecotype (or geographic origin) if comparisons are being made with other biological control programs using the same parasitoid species.
- Consider testing species of special economic or conservation interest (or their congeners).
- Conduct well replicated host ranges tests with controls under conditions that optimize the physiological condition of both test species and parasitoids, and provide standardized test conditions.
- Standardize host-parasitoid ratios and exposure times, choosing conditions that give high levels of parasitism in the target host as a basis for comparison with test species.
- Use no-choice tests initially for a conservative test; choice tests can contribute different information but probably are less informative than for weed biological control agents.

- When dissecting hosts to record parasitism, make detailed records of the reproductive status/fecundity of hosts, the incidence of superparasitism, and any evidence of a host immune response (such as melanization or abnormal development of parasitoid immature stages).
- Test laboratory predictions of non-target parasitism in post-release field studies.

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CHAPTER 10. EVALUATION OF LILY LEAF BEETLE PARASITOIDS FOR NORTH AMERICAN INTRODUCTION

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INTRODUCTION

The lily leaf beetle, *Lilioceris lilii* (Scopoli) (Coleoptera: Chrysomelidae), has spread into five Canadian provinces and six states in the northeastern USA in the 60 years since its introduction into North America. It is a serious pest of native and ornamental lilies and a candidate for classical biological control given that it is well regulated by a complex of seven parasitoids in Europe. However, unlike many of the key agricultural and forest pests, the arrival of this pest is not regarded as a disaster (except by lily growers). This has afforded us the opportunity to carefully evaluate host specificity using many different approaches without the serious time constraints that affect many programs. We have evaluated the parasitoid complex through field and laboratory tests with congeneric species in Europe; with studies of chemical ecology of the pests, their parasitoids, and their host plants in Europe; and with laboratory host range testing in the USA. These multiple approaches yield generally supportive, but sometimes contradictory results, which provide useful insight into the value and interpretation of these techniques.

LILIOCERIS LILII

The first published record of *L. lilii* in North America was by Brown (1946), who found it in Montreal, Canada, in 1945. The beetle, which had been found on Montreal Island as early as 1943 (LeSage, 1992), apparently did not cross the St. Lawrence River until 1978. Within three years, the beetle was found in Ottawa (140 km distant). It was subsequently found in Wellington, Nova Scotia, in 1992 (LeSage, 1992); Boston, Massachusetts, in 1992 (Livingston, 1996); Toronto, Ontario in 1993 (Gooderham, 1993); Portage la Prairie, Manitoba, in 1999 (LeSage, pers. comm); and Fredericton, New Brunswick, in 2002 (LeSage, pers. comm.). Since its discovery in Boston, the beetle has spread throughout New England and into northern New York.

The genus *Lilioceris* contains 142 species, of which 35 are found in the holarctic region, 60 are Oriental, 16 Australian, 20 Ethiopian, three neotropical, and the remaining eight species are of unknown distribution (Berti and Rapilly, 1976). Among the European species, *Lilioceris lilii* (Scopoli) 1863 appears to be the most widely distributed, with specimens recorded from as far north as Siberia and south through North Africa (Livingston. 1996). Berti and Rapilly (1976) trace the origin of *L. lilii* to the Orient. Lu and Casagrande (1998) and Yu *et al.* (2001) report the insect to occur in China.

This univoltine beetle overwinters as an adult and after initiating feeding in the spring, oviposits on the undersides of lily leaves. Larvae, which carry a fecal shield, pass through four instars before pupating in the soil. In North America, larval feeding often results in severe defoliation of cultivated *Lilium* and *Fritillaria* species as well as native lilies (Livingston, 1996). There are 21 species of lilies native to North America, including three *(Lilium canadense* L., *Lilium philadelphicum* L., and *Lilium superbum* L.) that lie within the eastern North American range of *L. lilii* (Woodcock and Stearn, 1943).

In Europe, the beetle is widespread and relatively common but seldom achieves pest status except in the United Kingdom, where it is an exotic species (Salisbury, 2003), and in some ornamental plantings in continental Europe, where natural enemies are likely disturbed by cultural practices such as bulb removal in winter (Kenis *et al.*, 2003).

NATURAL ENEMIES

No insect natural enemies have been reported on *L. lilii* in North America (LeSage, 1992; Livingston, 1996; Gold, 2004). In Europe, Gold *et al.* (2001) surveyed France and Switzerland and identified four larval parasitoids of *L. lilii*: *Tetrastichus setifer* Thomson (Hymenoptera: Eulophidae), *Lemophagus pulcher* Szepligeti (Hymenoptera: Ichneumonidae), *Lemophagus errabundus* Gravenhorst (Hymenoptera: Ichneumonidae), and *Diaparsis jucunda* (Holmgren) (Hym: Ichneumonidae). Haye and Kenis (2004) subsequently reported the occurrence of an egg parasitoid, *Anaphes* sp. (Hymenoptera: Mymaridae), reared from *L. lilii*, along with two tachinid flies attacking larvae, *Meigenia simplex* Tschorsnig and Herting, and *Meigenia uncinata* Mesnil (Diptera: Tachinidae). These three species are not being considered as potential biological control agents because the tachinids are known from other, unrelated hosts and *Anaphes* sp. needs to overwinter in an alternate host in order to complete its development (Haye and Kenis, 2004).

Tetrastichus setifer is the most widely distributed of the European parasitoids of *L. lilii*. In our surveys, it was found from the United Kingdom to Bulgaria and from northern Germany to Italy (Kenis *et al.*, 2002; Haye and Kenis, 2004). It is also known from the Czech Republic, Slovakia, France, the former Yugoslavia, and Sweden (de V. Graham 1991). *Tetrastichus setifer* is a gregarious species, averaging 7 parasitoid larvae per host (range = 2 to 26). It is univoltine, and mature larvae overwinter in the host's cocoon in the soil. Adult emergence is protracted over a period of several weeks in the spring. Females oviposit in all four larval stages of *L. lilii* (Haye and Kenis, 2004).

Diaparsis jucunda was reported by Horstmann (1971) from Sweden, Finland, Denmark, Germany, and the Czech Republic. Haye and Kenis (2004) found it to be the dominant parasitoid of *L. lilii* in central and southern Europe (Switzerland, Austria, Italy) on both cultivated and wild lilies. This species is nearly absent from western and northern Europe. This solitary larval parasitoid attacks all larval stages of *L. lilii* and kills the pre-pupa in the host cocoon, where it overwinters as a larva (Haye and Kenis, 2004).

Lemophagus errabundus was described by Gravenhorst in 1829 from Germany and was reported to attack *Lilioceris merdigera* (L.) in France (Elliott and Morley, 1911). Haye and Kenis (2004) found it to displace *D. jucunda* as the dominant parasitoid in western and northern Europe (United Kingdom, Netherlands, western France, and northern Germany), but it is rare elsewhere. This solitary, univoltine larval parasitoid kills the beetle in the pre-pupal stage and overwinters as a teneral adult in the host cocoon.

Lemophagus pulcher, first described from Hungary, was found by Kenis et al. (2002) and Haye and Kenis (2004) to be widespread, occurring in nearly all regions investigated (except the United Kingdom), but dominating only in Bulgaria. It is very similar to *L. errabundus*, but 4-58% of the individuals emerge for a second generation when parasitized larvae are reared in the laboratory, and there are evidences that a partial second generation also occurs in the field. This species is commonly attacked by the hyperparasitoid *Mesochorus lilioceriphilus* Schwenke, and hyperparasitism rates of 30% are common. *M. lilioceriphilus* also occasionally attacks *Lemophagus errabundus.*

Parasitism rates of *L. lilii* are generally high throughout Europe, with averages of 25-78% (Haye and Kenis, 2004). Different parasitoids predominate in different regions and at different times of the season (Haye, 2000; Kenis *et al.*, 2002; Kenis and Haye 2004).

OTHER HOST SPECIES IN EUROPE

Berti and Rapilly (1976) report six species of *Lilioceris* in Europe, but only three are known from western and Central Europe. *Lilioceris merdigera* L. is a widespread species, feeding on *Polygonatum multiflorum* L., *Polygonatum verticillatum* (L.) *Polygonatum odoratum* (Miller), *Convallaria majalis* L., *Allium ursinum* L., and in gardens on chive (*Allium schoenoprasum* L.) (Haye and Kenis, 2004). *Lilioceris tibialis* (Villa) is a rare species found in the Alps that feeds on wild *Lilium martagon* L. and *Lilium bulbiferum* L. (Haye and Kenis 2004). These congeneric species can serve as hosts for the same parasitoids as *L. lilii*, and they were used to evaluate host range of the parasitoids found on *L. lilii.* There is no record of the dominant parasitoids of *L. lilii* (*T. setifer, D. jucunda, L. errabundus, L. pulcher*) attacking other hosts in Europe.

In addition to *L. lilii*, three other criocerid beetles have become important pests in North America, and these species have been subjected to extensive biological control research: the cereal leaf beetle, *Oulema melanopus* (L.); the common asparagus beetle, *Crioceris asparagii* (L.); and the spotted asparagus beetle, *Crioceris duodecimpunctata* (L.). A complex of European parasitoids of the cereal leaf beetle has been established in North America, including *Tetrastichus julis* (Walker), *Lemophagus curtus* Townes, *Diaparsis temporalis* Horstmann, and *Anaphes flavipes* (Foerster) (Haynes and Gage, 1981). The introduced asparagus beetles also have European parasitoids, including *Tetrastichus asparagi* Crawford and *Lemophagus crioceritor*

Aubert, both of which were released in North America against *C. asparagi*; and *Tetrastichus crioceridis* Graham and *Diaparsis truncatus* (Gravenhorst), which were released against *C. duodecimpunctata* (Hendrickson *et al.*, 1991). Despite the extensive collection and rearing of the European parasitoids of these species for these biological control programs, none of the parasitoids of *Lilioceris* species were reported from these hosts.

RELATED BEETLES IN NORTH AMERICA

Lilioceris lilii is among the 1,720 North American species in the family Chrysomelidae, which are divided among 195 genera (Triplehorn and Johnson, 2005). North American species are grouped in 11 subfamilies, including Criocerinae, which includes the genera *Crioceris, Oulema, Neolema, Lilioceris*, and *Lema* (Triplehorn and Johnson, 2005).

The only insects in North American in the genus *Crioceris* are the introduced asparagus beetles *C. asparagi* and *C. duodecimpunctata* (Arnett, 2000). *Oulema* is represented by at least 10 species, including the European cereal leaf beetle, *O. melanopus*. About 15 species of *Lema* are known to occur in the eastern and southern United States, including *Lema trilineata* White, which feeds on potatoes and other solanaceous plants (Triplehorn and Johnson, 2005). *Neolema* contains at least four North American species, including *Neolema sexpunctata* (Oliver), which feeds on the common dayflower, *Commelina communis* L. The lily leaf beetle, *L. lilii*, is the only species of *Lilioceris* that presently is known to exist in North America.

RESEARCH RATIONALE

We evaluated four parasitoids for possible introduction against *L. lilii* in North America: *Tetrastichus setifer, Diaparsis jucunda, Lemophagus errabundus,* and *Lemophagus pulcher*. All four of these species were found to cause high levels of parasitism in various locations in Europe, and unlike the tachinid species, they appeared to have reasonable host specificity.

Host specificity research in Europe concentrated on the congeneric beetles *L. merdigera, L. tibialis,* and the pest itself, *L. lilii*. These three species are the only *Lilioceris* species occurring in western and Central Europe. They all have a similar biology and ecology, and we presumed that if parasitoids proposed for introduction distinguished among these congeneric species in Europe, they would also do so among potential U.S. hosts related to *L. lilii* at more distant taxonomic and ecological levels. Thus, we believed that a parasitoid of *L. lilii* that would not attack *Lilioceris merdigera* and *L. tibialis* would be highly unlikely to attack a species from another genus.

The chemical ecology research in Europe attempted to elucidate the stimuli for attraction and oviposition of the four key parasitoids of *L. lilii* using the three *Lilioceris* species and their host plants, as well as extracts from *L. trilineata* from North America. The rationale for this research in the context of host specificity is that we expected to find stimuli that were specific to the lily/*Lilioceris* system whose absence would preclude the use of other species as hosts.

Further host range testing was conducted in quarantine in North America to expand upon the work with congeneric species done in Europe and determine if parasitoids were specific at the genus level. *Lilioceris lilii* is the only North American insect in its genus, and it arrived relatively recently. Thus parasitoids with genus-level specificity could be safely released without fear of affecting other insect populations. In selecting potential hosts, we focused upon the most closely related species, attempting to get at least one species from each of the North American genera within the Criocerinae. Specifically, we selected *Oulema melanopus, Crioceris asparagi,* and *Lema trilineata* for our tests. We would have evaluated *Neolema sexpunctata,* but we were unable to collect this species in numbers adequate for experimentation. We broadened the taxonomic scope of our tests by including three additional chrysomelids: the imported willow leaf beetle (*Plagiodera versicolora* Laicharting), the Colorado potato beetle (*Leptinotarsa decemilineata* [Say]), and two *Galerucella* species introduced for biological control of purple loosestrife (*Lythrum salicaria* L.). Additionally, we tested one coccinellid: the Mexican bean beetle (*Epilachna varivestis* Mulsant). These non-criocerid test species were selected based upon availability and relative ease of rearing. The laboratory experiments required growing (or collecting) the host plants for the test species and inducing these beetles to oviposit so that non-parasitized larvae were available at the time that *L. lilii* and its parasitoids were available for testing. Our laboratory host preference tests were expanded to include parasitoid oviposition response to previously parasitized *L. lilii* larvae to test for cleptoparasitism and to help evaluate some of the field results observed in Europe.

STUDIES ON CONGENERIC BEETLE SPECIES

Methods

Two different investigations were conducted in Europe: (1) evaluation of sympatric populations in the field and (2) laboratory host specificity screening.

Sympatric populations This research was conducted at CABI Bioscience in Switzerland by Claire Scarborough, working under the direction of Marc Kenis. She collected third and fourth instar larvae of various *Lilioceris* species between May and July, 2002, from four natural sites in the Jura region of Switzerland (Scarborough, 2002). All four sites had sympatric populations (separated by less than 500 m) of the beetle *L. lilii* feeding on *Lilium martagon* and the beetle *L. merdigera* feeding on *P. multiflorm* and *P. verticillatum.* At a fifth site, situated in the Alps, sympatric populations of the beetles *L. lilii* and *L. tibialis* were found feeding on the lily *L. martagon.*

Larvae from all sites were reared on excised host plants in 1.3 liter plastic containers with a bottom layer of wet fine vermiculite and allowed to pupate. After emergence of adult beetles and some non-diapausing parasitoids, the containers were sifted and the parasitoids that had emerged from beetles were identified based on cocoon features and adult emergence

Host specificity screening These tests were carried out by Haye (2000), Kenis et al. (2001, 2002), and Scarborough (2002). Laboratory rearing of the three species was set up in cages using adults or eggs collected from field populations in Switzerland. Larvae were fed with cultivated lily (for *L. lilii* and *L. tibialis*) and cultivated onion (for *L. merdigera*)

Parasitoids used in these experiments (*L. pulcher, L. errabundus, D. jucunda,* and *T. setifer*) were reared from cocoons collected in previous years and held over winter at 2°C. The cocoons, held in Petri dishes in polystyrene boxes lined with damp cellulose paper, were moved to room temperature (20-24°C) and monitored daily for adult emergence. For the ichneumonid species, males of a single species were held together in 1.3 liter containers in groups of four or five and provided with moist cotton wool dipped in honey. Females were placed in cages with males for approximately 24 hours for mating and then held separately for another 24 hours before use in experiments. Between tests, parasitoids were kept in incubators at 11-17°C, 16:8 L:D photoperiod and ambient humidity, with access to moist cotton wool and honey.

In choice tests, three larvae of *L. lilii* and three larvae of either *L. merdigera* or *L. tibialis* were placed in a 9.4 cm diameter Petri dish and one parasitoid was introduced for ten minutes, during which time ovipositions on individual larvae were directly observed. Because the eulophid *T. setifer* oviposits for up to 30 minutes compared to a few seconds for the three ichneumonids, experiments with *T. sertifer* were run for 3 hours. Following each test, larvae were reared on their proper host plants and held over wet fine vermiculite in 0.15 liter containers until they were dissected to determine parasitism.

In no-choice tests, a single female was introduced into a dish of its dominant host (typically *L. lilii*) and observed for 10 minutes to count ovipositions. She was removed and allowed 10 minutes before a second exposure to three larvae of the alternate host. Again, ovipositions were recorded during this second exposure, after which the female was provided a second 10-minute rest. A third 10-minute exposure to the initial test species was conducted to confirm her ability (or willingness) to oviposit. Exposed beetle larvae were reared over wet, fine vermiculite before dissection to determine parasitism.

STUDIES WITH CONGENERIC BEETLES: RESULTS AND DISCUSSION

Sympatric populations Scarborough (2002) found high parasitism among the natural sympatric populations of *L. lilii* and *L. merdigera* (86.1% and 79.2%, respectively, among the total 957 *L. lilii* and *524 L merdigera* larvae that she collected). *Diaparsis jucunda* was the principal parasitoid of *L. lilii*, accounting for 142 of 198 (71.1%) parasitoids recovered from that host, but it accounted for only 10% of the total parasitism of *L. merdigera* (8 of 80 recovered). Conversely, the *Lemophagus* species (principally *L. pulcher*) were more prevalent in *L. merdigera* than in *L. lilii*, accounting for 71 of 80 (87.6%) parasitized larvae vs. 56 of 198 (28.3%), respectively. *Tetrastichus setifer* was found only in one *L. merdigera* (1.2% of total).

At the site with *L. tibialis* and *L. lilii* in the western Alps, Scarborough found 98.3% parasitism of 88 *L. lilii* larvae, but only 29.3% parasitism of 527 larvae of *L. tibialis.* As with the other sites, *D. jucunda* was most common in *L. lilii*, accounting for 38 of the 45 parasitized larvae from that host (84.4%) vs. 2.7% (3 of 111) parasitized larvae of *L. tibialis*). *Lemophagus* species were more common in *L. tibialis* than *L. lilii*, comprising 77 (69.4%) of the 111 parasitized larvae of *L. tibialis* vs. 2 (4.4%) of the 45 parasitized larvae of *L. lilli.* At this site, *T. setifer* was relatively common, accounting for 27.9% (31/111) of total parasitism of *L. tibialis* and 11.1% (5/45) of the parasitized *L. lilii* larvae.

These results are consistent with those observed by Haye and Kenis (2004) in non-sympatric populations at natural sites on wild plants in Switzerland. Although all of the four major parasitoids occasionally attack all three congeneric hosts in natural settings, strong host preferences are shown. *L. lilii* is mainly attacked *by D. jucunda*, which is found in very low numbers on the two other hosts and only in the vicinity *of L. lilii* populations. *L. pulcher* is by far the main parasitoid of *L. merdigera. T. setifer* has been observed in high numbers attacking populations of *L. tibialis,* and *L. errabundus* is found occasionally on all the three hosts. These strong host preferences shown in natural habitats in Switzerland do not necessarily reflect their potential as biological control agents, given that all four parasitoids have been found as dominant parasitoids of *L. lilii* in gardens in different regions of Europe (Haye and Kenis, 2004).

Host specificity screening All four parasitoids were successfully reared on the three host species. In choice tests with pairs of species, *L. pulcher* attacked *L. tibialis* and *L. merdigera* as readily as *L. lilii,* and this response was not affected by the parasitoid's rearing host (Scarborough, 2002). Eggs and larvae were found in all three hosts, supporting field observations that all three congeners are adequate hosts for this species.

Lemophagus errabundus also readily attacked both *L. lilii* and *L. tibialis* in choice tests with species pairs, but it demonstrated a borderline significance ($P= 0.07$) in preference for *L. lilii* over *L. merdigera,* a preference that was supported by no-choice tests in which significantly fewer ($P = 0.004$) larvae of *L. merdigera* were selected for oviposition compared to *L. lilii.*

Contradictory results were obtained with *Diaparsis jucunda.* Haye (2000) and Kenis et al. (2001) observed that *D. jucunda* showed a strong preference for *L. lilii* in choice-tests ($P<0.001$). In no-choice tests, it oviposited in *L. lilii* very frequently, whereas ovipositions in *L. merdigera* occurred at much lower frequency. In contrast, two years later, Scarborough (2002) observed *D. jucunda* attacking *L. tibialis* and *L. merdigera* as readily as *L. lilii.* The parasitoid's rearing host did not affect the parasitoid's oviposition preferences.

Tetrastichus setifer reared from *L. lilii* spent significantly more time on *L. lilii* than on *L. tibialis* in paired choice tests ($P = 0.009$) and showed a significant preference for *L. lilii* over *L. merdigera* ($P = 0.0002$). However *T. setifer* reared from *L. tibialis* showed no preference between that host and *L. lilii.*

Collectively, these screening studies indicate that all three beetle species are attacked by all four parasitoid species. The preference for particular hosts observed in sympatric populations in the field did not clearly appear in the laboratory tests. The contradictory results obtained with *D. jucunda,* perhaps due to the use of different parasitoid populations, emphasize the need for large sample sizes and replicates with different strains in such screening tests.

STUDIES ON CHEMICAL SCREENING

METHODS

The research on the chemical ecology of the parasitoids of *L. lilii* has been led by Dr. Urs Schaffner at the CABI Bioscience Switzerland Centre. Both olfactometer bioassays and contact bioassays were used in this research. The olfactometer tests used a round, static-air, four-chamber olfactometer (Steidel and Schöller, 1997) in which the test substance was placed at random in one or two of the chambers while the other two or three chambers remained empty. A single parasitoid was released onto a fine mesh screen over these four chambers and the time spent over the test chamber and the three controls was recorded during a five-minute assay period. Contact bioassays were conducted in 9 cm glass Petri dishes in which two or three substrates placed equidistant from one another were offered simultaneously to a single wasp placed in the center of the arena. Contact frequency, contact duration, and frequency of ovipositor probing were recorded for five-minute periods.

Tested substrates included, among others, *L. lilii* larvae with or without their fecal shield; fecal shield of *L. lilii* alone; shield extracts of *L. lilii*, *L. merdigera* and the North American non-host *Lema trilineata* on paper dummies; lily leaves damaged by *L. lilii*, by other defoliators or artificially damaged; *Polygonatum verticillatum* leaves damaged by *L. merdigera*; and larvae and fecal shield of the cereal leaf beetle, *Oulema melanopus*.

All four larval parasitoids were tested. However, *L. errabundus* did not respond consistently to the contact bioassays, and both *L. errabundus* and *T. setifer* were unresponsive to the olfactometer bioassays. Therefore, most tests were carried out with the more cooperative *L. pulcher* and *D. jucunda*. When possible, naïve and experienced females were compared in their response to signal sources. The experimental approaches and results are described in detail in various publications and unpublished reports (Schaffner and Kenis, 1999; Kenis *et al.*, 2001; Schaffner and Müller, 2001; Scarborough, 2002; Schaffner, 2002). Only a summary of the most relevant results is presented herein.

CHEMICAL SCREENING RESULTS AND DISCUSSION

In both olfactometer and contact bioassays, *L. pulcher* was found to be attracted to *L. lilii* larvae with and without their fecal shields, to the fecal shields alone, to shield extracts on dummies, and to lily leaves that had been damaged by *L. lilii*. Females were induced to oviposit on dummies by shield extracts. Larvae and the fecal shield of *O. melanopus* and fecal shield extracts *of L. trilineata* were found to be significantly attractive *to L. pulcher.*

Diaparsis jucunda responded rather similarly, being attracted to fecal shields with and without larvae and lily foliage that had been damaged by *L. lilii* larvae, and being stimulated to oviposit by extracts from the fecal shields of *L. lilii* larvae. *Diaparsis jucunda* showed ovipositor probing on dummies with shield extracts of *L. trilineata* but only a nonsignificant preference for such dummies over untreated controls. Interestingly, *D. jucunda* was not attracted to *P. verticillatum* leaves damaged by *L. merdigera.* In contrast, it was attracted to dummies treated with fecal extracts from *L. merdigera*, displaying ovipositor probing (*L. pulcher* was not tested with *L. merdigera* extracts).

Scarborough (2002) also investigated in these chemical screening experiments the effect of prior experience on host selection. She found that, while *L. pulcher* host selection behavior is largely innate, it may change with experience. Naïve *L. pulcher* females did not probe a dummy treated with an extract of *L. lilii* fecal shield, but after experience with *L. lilii* larvae, the females showed increased frequency of probing and increased duration of contact with the dummy. In contrast, *D. jucunda* host specificity appears fixed regardless of experience: both naïve and experienced females respond to fecal extracts of *L. lilii* with frequent ovipositor probing and prolonged contact with the larva. These observations may indicate greater host plasticity in *L. pulcher* given that its behavior may change with experience. In contrast, the innate host-selection behavior of *D. jucunda* suggests a narrower host range.

Tetrastichus setifer females also responded positively to fecal shields and fecal shield extracts of L. lilii. When presented with *L. lilii* and *L merdigera* larvae, they were found to spend less time on the *L. merdigera* larvae in contact bioassays. When the fecal shields of these hosts were switched (putting *L. merdigera* feces on *L. lilii* and vice- versa), the parasitoids switched their preference, spending significantly more time on *L. merdigera* (Scarborough, 2002).

Overall, these experiments, while incomplete, were useful in assessing host preference. *Lemophagus pulcher*, while attracted to *L. lilii*, its fecal material, and its damaged host plants, also was attracted to, and oviposited in, dummies treated with extracts from *L. trilineata*, a North American insect in a different genus from the normal host. *Lemophagus pulcher* also demonstrated a greater plasticity in host response based upon prior experience than *D. jucunda*. These results, combined with the field results of sympatric populations and laboratory host screening tests with congeneric species, indicate that special attention might be given this parasitoid in further host specificity studies. *Diaparsis jucunda* showed generally similar responses to those of *L. pulcher*, including a non-significant positive response to *L. trilineata*. *Tetrastichus setifer* showed selectiveness in fecal shield attractiveness – responding more strongly to fecal material from *L. lilii* than to that from *L. merdigera*. This is consistent with the laboratory screening with intact larvae.

Like the congeneric studies, the chemical screening tests indicate possible host preferences in some species, but they do not identify any of the four parasitoids as host specific to a particular species. Furthermore, one parasitoid (*T. setifer*) responded in only the contact bioassay and another (*L. errabundus*) did not respond in either test. It is likely that, with additional experimentation, it would be possible to establish test conditions that allowed these species to respond, but this problem brings into question the general utility of this approach to host specificity screening.

TESTS IN QUARANTINE

Source and Rearing of Parasitoids

Parasitoids used in these experiments were reared from *L. lilii* larvae collected in Europe. In 1998, these were collected in northwestern France (Gold et al. 2001), and in subsequent years, they were collected throughout Europe (Haye and Kenis, 2000; Gold, 2004). Field-collected larvae were held in 1.4 l plastic containers under laboratory conditions (ca 25°C) and fed lily leaves until cocoon formation. Resultant cocoons were then held under similar conditions until all adult *L. lilii* emerged. Parasitized cocoons were then held at 4°C in a growth chamber for a minimum of two months before shipment in chilled containers to the URI Biological Control Laboratory. In our quarantine laboratory, parasitoids were held at 4°C until needed for experiments and then warmed to 25°C for adult emergence. From 1999-2003, 12,978 parasitized *L. lilii* cocoons were shipped to URI, including 4,352 *T. setifer*, 4,895 *D. jucunda* and 3,731 *Lemophagus* spp. Parasitoids that emerged were used in research. The remaining cocoons were dissected and information on species was provided to Marc Kenis at CABI in Switzerland for parasitoid distribution surveys. Only field-collected parasitoids were used in our host specificity studies.

We maintained the pest beetle *L. lilii* in quarantine at the URI Biological Laboratory in a colony that was started (and periodically refreshed) with adults collected near Boston, Massachusetts. Beetles were reared on potted Asiatic and Oriental lilies grown from organically produced bulbs in a greenhouse under ambient temperature conditions and a minimum of 16h daylight, supplemented by 400 watt sodium vapor or 1000 watt mercury vapor lights on timers. In the laboratory, beetles were reared in screen cages (45 cm on a side) under fluorescent lights with a 16:8 (L:D) photoperiod. Newly emerged adult beetles were fed for a minimum of one week and then stored in plastic freezer cartons with paper towels in a refrigerator at 7°C for three months, after which they were removed and used in rearing (Gold, 2004).

HOST RANGE TESTS

Methods for host range tests Newly emerged adult parasitoids were held in 1.8 liter plastic jars in growth chambers under fluorescent lights with a 16:8 (L:D) photoperiod and a day:night temperature cycle of 20:15 °C. The jars were removed from the growth chambers for 4h during host specificity tests at ambient room temperature (25°C). These tests were conducted on a table next to a window with supplemental fluorescent lighting. Putative hosts evaluated in these experiments included the *Criocerinae* species *O. melanopus, C. asparagi,* and *L. trilineata.* We also tested three non-Criocerinae chrysomelids: *P. versicolora*, *L. decemilineata*, and *Galerucella* sp. and the coccinellid *E. varivestis.*

Test larvae were placed on stems of their host plant for a minimum of 2h before exposing them to parasitoids in all experiments because Schaffner and Müller (2001) showed that some species of *L. lilii* parasitoids are attracted to plants damaged by *L. lilii* larvae. For these feeding periods and subsequent parasitoid exposures, 10-12 second or third instar larvae were placed on an excised stem of a host plant, and that stem was placed in a water pic filled with tap water. In the tests with ichneumonid species, one to five female wasps (generally three, rarely one) and one male wasp were placed in a jar with the test larvae for 2 hours. In the tests with eulophid species, ten females and at least one male *T. setifer* were placed in a jar for 2 hours. Wasps were provided water and honey with either a damp wick in a water pic and a streak of honey or honey water on a wick. Immediately after exposure to the test larvae, the same parasitoid adults were given a second exposure to 10-12 second or third instar *L. lilii* larvae on a lily stem using the same protocol as above. When parasitism was found in a test larva, as well as in the subsequent test with lily leaf beetle larvae, the results were analyzed using a Chi-square test (Johnson and Bhattacharyya, 1987).

After parasitoid exposure, larvae were reared in 240 ml plastic containers with a bottom layer of 50 cc of damp vermiculite and fed leaves of the host plant for approximately ten days before they were dissected to determine parasitism. In all experiments, the first exposure of a female parasitoid was to a nontarget test species (other than *L. lilii*), and these exposure data were used only if parasitoids successfully attacked *L. lilii* larvae after that first exposure. Depending upon the parasitoid species, between 35% and 71% of the tests were rejected because of lack of attack on *L. lilii*, involving well over 1,500 test larvae and an equivalent number of *L. lilii.* Among the possible 32 tests (8 test larvae x 4 parasitoid species) we obtained useful results (with positive results in controls) in 27 combinations with an average of 35.6 test larvae per test. The *L. lilii* controls in these tests averaged 27.3% parasitism.

Results from Host Range Tests Among the ichneumonids, Gold (2004) found that neither *D. jucunda* nor *L. errabundus* oviposited in any of the eight nontarget hosts tested. *Lemophagus pulcher* oviposited in two nontarget insects, *L. trilineata* and *C. asparagi.* We found 6 of 76 (7.8%) *C. asparagi* larvae were parasitized by *L. pulcher* in a test where the controls showed 30 out of 102 (29.4%) parasitized. A significant difference in these ratios (Chi-square test, $P = 0.001$) indicates a preference for *L. lilii* over *C. asparagi. Lemophagus pulcher* parasitized 11 of 33 *L. trilineata* (33%) vs. 9 of 35 *L. lilii* (25.7%). This non-significant difference (Chi-square test, $P = 0.30$) indicates that *L. trilineata* is as acceptable as *L. lilii* to this parasitoid.

None of the putative hosts exposed to *T. setifer* were attacked except a single larva of *L. trilineata*, which was found to contain *T. setifer* larvae. The parasitoid ratio (1/73) was significantly different (Chi-square test, $P = 0.001$) from the parasitism of the *L. lilii* control in this test (15/63), indicating a distinct preference of *L. lilii* as a host by this species. Gold (2004) also conducted preliminary tests in which *T. setifer* was exposed to *L. trilineata* using a slightly different protocol, and in those tests 0 of 79 larvae were parasitized. We consider the parasitism of a single *L. trilineata* larva out of 150 tested to be an anomaly, perhaps due to confinement in too small a container.

HOST PREFERENCE TESTS (PARASITIZED VS. NON-PARASITIZED)

Methods for host preference tests (after Gold, 2004) We assessed the behavior of parasitoids exposed to previously parasitized hosts in a series of choice tests conducted in 8.5 cm diameter Petri dishes. To obtain larvae stung by the ichneumonid wasps, we placed three second or third instar *L. lilii* larvae on lily leaf fragments in a covered Petri dish. Individual female wasps were placed in the Petri dish, and the larvae were removed once they were stung. For *L. errabundus, L. pulcher,* and *D. jucunda*, a sting entailed insertion of the ovipositor for a minimum of two, two, and three seconds, respectively (Haye and Kenis, 2000). Because of the long oviposition time of *T. setifer*, we used a different protocol to obtain stung larvae. We placed 20 female wasps in a Petri dish with ten second or third instar lily leaf beetle larvae. Larvae were removed from the dish once they had been stung, which in this case was defined as insertion of the ovipositor for at least 15 minutes, exceeding the 13 minute minimum oviposition requirement reported by Haye and Kenis (2000).

Choice tests were conducted 24 hours after the larvae were stung. In the choice tests with the ichneumonid parasitoids, three *T. setifer*-stung larvae and three unstung larvae were placed alternatively in a circle on fragments of lily leaf in an 8.5 cm Petri dish. An individual female wasp was placed in the Petri dish for 15 minutes. Every ovipositor insertion was recorded. When the tests were conducted with *T. setifer*, ten female wasps were placed in a Petri dish with three ichneumonid-stung and three unstung lily leaf beetle larvae for 15 minutes. Total ovipositor insertions of all ten females were recorded. Trials were replicated six to ten times, depending upon the availability of wasps and host insects. New females and a clean Petri dish were used for each replicate. All results were analyzed with the Wilcoxon matched-pairs signed-ranks test (Johnson and Bhattacharyya, 1987). Larvae were dissected after approximately ten days to determine parasitism, including which parasitoid survived in cases of multiple-species ovipositions.

In a second series of choice tests, a similar protocol was followed except that the choice exposure was conducted within three hours after the first exposure instead of 24 hours later. Results were again analyzed with the Wilcoxon matched-pairs signed rank test, and larvae were dissected after approximately ten days.

Results for host preference tests Gold (2004) found that *L. errabundus* does not distinguish between *L. lilii* larvae that were and were not previously stung by *T. setifer* or *L. errabundus* in tests conducted 3 and 24 hours after initial parasitism. In three of four trials, the same applied in the reverse direction: *Tetrastichus setifer* did not distinguish between larvae that were and were not previously stung by *L. errabundus.* However, in one test, *T. setifer* stung significantly more *L. lilii* larvae (3.7 vs. 2.0) that were previously stung by *L. errabundus.* In one of two tests, *T. setifer* showed a significant preference for unstung larvae vs. those that were previously stung by *D. jucunda* (2.3 vs. 1.0). *Diaparsis jucunda* preferred unstung larvae over those previously parasitized by *T. setifer* in one of three trials, and *L. pulcher* did not distinguish between larvae that were and were not previously by *T. setifer* in a single trial.

Although Gold (2004) did not test all possible combinations of parasitoids, she did test all four species under evaluation and found no indication of cleptoparasitic tendencies in any of them. There is also little or no indication that the parasitoids distinguish between previously parasitized and unparasitized larvae – even among those parasitized by their same species (Gold, 2004). Tests conducted 3 hours after initial parasitoid exposure gave results similar to the exposures conducted 24 hours later. It is possible that this test protocol could have masked behavior that occurs in the field. Following oviposition, female ichneumonids, particularly *L. errabundus,* are frequently observed dragging their abdomens across the leaf on which the parasitized larva resides, possibly marking these leaves as containing parasitized larvae. Our protocol involved using new leaves and clean Petri dishes for each exposure, thereby removing any signals that were not directly associated with the larva.

In dissecting the parasitized *L. lilii* larvae that resulted from the behavior experiments, Gold (2004) found that when *T. setifer* oviposits first, it is more likely to survive and develop in lily leaf beetle larvae than are *D. jucunda, L. errabundus,* or *L. pulcher.* However, if *L. errabundus* stings the lily leaf beetle first, either *T. setifer* or *L. errabundus* may survive; and when *D. jucunda* stings first, it is more likely to survive and develop than *T. setifer.*

SUMMARY

The three types of investigations conducted (field studies of congeneric species under sympatry, chemical ecology, and laboratory screening) all provided useful results, which together present a clear picture of host specificity in parasitoids of *L. lilii.*

Studies of sympatric populations of *L. lilii* and its congeners showed *D. jucunda* to be the most discriminating of the four primary parasitoids of *L. lilii,* demonstrating a strong preference for *L. lilii* over *L. merdigera* or *L. tibialis.* Laboratory chemical screening results strengthened this observation. *Diaparsis jucunda* is attracted to lily foliage that has been damaged by *L.*

lilii larvae, and it is stimulated to oviposit by extracts from the fecal shields of *L. lilii* larvae (Scarborough, 2002). Further, this species is not attracted to *P. verticillatum* leaves damaged by *L. merdigera.* Studies in quarantine showed that *D. jucunda* does not attack any of the eight nontarget test species presented in tests in which *D. jucunda* consistently parasitized *L. lilii.* Furthermore, *D. jucunda* is not a cleptoparasitoid. In competition with *T. setifer* within a *L. lilii* larva, we found that whichever species attacked was most likely to survive. The only negative results with *D. jucunda* are reported by Scarborough (2002), who determined that it attacked *L. tibialis* and *L. merdigera* as readily as *L. lilii* in choice tests based on pairs of species presented in Petri dishes. This may have resulted from the choice of experimental chambers. Livingston (1996) obtained anomalous results when she confined the cereal leaf beetle parasitoid *Anaphes flavipes* (Foerster) with lily leaf beetle eggs in a Petri dish without host plants. Although *Anaphes* readily attacked *L. lilii* under these conditions (and Livingston reared them for several generations in this manner), the parasitoid failed to attack these eggs in larger screened cages (45 cm on a side) in the laboratory or under field conditions.

Lemophagus pulcher was shown to be less host specific with all three approaches. In natural sympatric populations with *L. lilii* congeners, *L. pulcher* was more common in *L. merdigera* and *L. tibialis* than in *L. lilii.* Laboratory chemical screening tests with these congeners and their host plants showed that *Lemophagus pulcher* is attracted to *L. lilii,* its fecal material, and its damaged host plants. It also shows attraction and oviposition responses to extracts from *Lema trilineata,* and it demonstrated a greater plasticity in host response based upon prior experience than *D. jucunda.* In choice tests with pairs of hosts, *L. pulcher* attacked both *L. tibialis* and *L. merdigera* as readily as *L. lilii* in Petri dishes, and eggs and larvae were found in all three hosts. Laboratory tests in quarantine showed that *L. pulcher* attacked *L. trilineata* as readily as *L. lilii,* and it also oviposited in *C. asparagi.* This species is clearly the least host specific of the four species under consideration, and despite the potential advantage of having a partial non-diapausing population, it is presently not under consideration for release in North America. It is not clear, however, that *L. pulcher* would attack *L. trilineata* if this wasp were released in North America because it also attacked *C. asparagi* in our laboratory tests, and from all indications, it does not attack this host in the field in Europe. It is common for laboratory tests to indicate a wider host range than actually occurs in the field (Federici and Maddox, 1996; Strand and Obrycki, 1996).

Lemophagus errabundus and *T. setifer* were somewhat intermediate in their responses in this battery of tests. Neither species was very common in the sympatric populations studied by Scarborough (2002), but both species have shown to be more common on cultivated *L. lilii* in other areas, such as western and northern Europe. In natural environments in Switzerland, *Tetrastichus setifer* was more common in *L. tibialis* than in *L. lilii.* Neither *L. errabundus* nor *T. setifer* responded to the olfactory bioassay test and only *T. setifer* responded in the contact bioassay where it was more attracted to fecal material from *L. lilii* than from *L. merdigera.* In laboratory screenings, *L. errabundus* showed a preference for the beetle *L. lilii* over *L. merdigera. Tetrastichus setifer* reared from *L. lilii* was more attracted to *L. lilii* than to *L. tibialis* or *L. merdigera.* However, *T. setifer* reared from *L. tibialis* showed no preference between that host and *L. lilii* (Scarborough, 2002). Finally, in quarantine studies with eight nontarget test species, *L. errabundus* attacked nothing but *L. lilii,* and the same was true for *T. setifer* (except for the single anomalous parasitized *L. trilineata* of 150 exposed). Thus, it appears that both *L.*

errabundus and *T. setifer* have host preferences within the genus *Lilioceris,* and they likely would not attack insects outside of that genus.

RECOMMENDATIONS

When species in the same genus as the target pests are found with sympatric populations, field sampling may be used to determine parasitoid preferences. Our studies suggested host preferences among the species, but fell short of showing any parasitoid to be host specific at the species level. On the other hand, if a parasitoid did attack only a single species among sympatric congeners (particularly on the same host plant), we could be quite certain that it would not attack more distantly related hosts. Given that our suvey was relatively inexpensive, it was probably well worthwhile. The same is true for laboratory screening of parasitoids on congeneric species – although such work is more time-consuming.

Chemical ecology studies provide useful insight into parasitoid behavior, but in this case, they were not specifically designed to evaluate host specificity. Theoretically, it would be easier to get fecal extracts from various criocerid hosts and evaluate them in olfactometers than to simultaneously rear various test species and the *L. lilii* controls and also have the right size host plants available when the parasitoids are in prime condition for oviposition. However, we probably need a larger body of evidence supporting this approach before we can substitute tests of this nature for the type of host range testing we did in quarantine. These studies do, however, contribute greatly to the growing body of knowledge about parasitoid behavior, and results will influence the design and interpretation of other laboratory experiments. For instance, it was quite clear from the work of Schaffner that the *L. lilii* parasitoids are generally attracted to lilies that are damaged by *L. lilii.* Thus, in all of our host range tests in quarantine, test species were confined on their host plants prior to the experiment and then kept on the same damaged plant during exposure to parasitoids. Since this is a very common phenomenon among parasitoids, it should be a standard practice for studies of host specificity. The chemical screening tests also showed parasitoid host preferences to be influenced by a parasitoid's prior host exposures. To be conservative, in our choice tests, parasitoids were first tested against the nontarget species (non-*L. lilii*) host and then tested on *L. lilii* to confirm parasitoid activity.

The choice of testing arena remains one of the key issues in laboratory testing. Our initial testing of *A. flavipes* on *L. lilii* eggs in Petri dishes gave completely spurious results. Based upon a high attack rate and successful rearing of this cereal leaf beetle parasitoid on *L. lilii* eggs, we attempted several unsuccessful field releases before determining that this parasitoid behaved differently in a large (45 cm on a side) laboratory cage with eggs on their proper host plant (Livingston, 1996). Scarborough (2002) also found *L. lilii* parasitoids to be relatively nondiscriminating among potential hosts when confined in Petri dishes. For example under such conditions, *D. jucunda* attacked *L. tibialis* and *L. merdigera* as readily as it did *L. lilii,* even though all other evidence pointed to a marked preference for *L. lilii* over the other two. Based upon these considerations, we used clear plastic jars (1.8 liter) with relatively large (12 cm) screw tops covered by screen. These 16 cm tall containers were large enough to house a host plant stem in a water pic and allow for parasitoid flight, but were small enough that we could readily follow the activity of the parasitoids. Exposures were conducted in front of large windows (out of direct sun), and parasitoids appeared to behave normally during these tests. Test results showed considerable selectivity toward the nontarget test species and a reasonably high level of parasitism in the *L. lilii* controls. Oviposition of the ichneumonid *L. pulcher* in the beetle *L. trilineata* was consistent with olfactometer tests and other tests indicating that this parasitoid is relatively non-specific. We do not have field tests or tests in larger cages to validate this experiment. We also observed that *L. pulcher* would occasionally attack *C. asparagi* in our 1.8 liter containers when this test species was confined on asparagus stems. In this case, it is likely that our test is not indicative of field results in Europe, where *C. asparagi* is attacked by a different parasitoid, *Lemophagus crioceritor* Aubert (Hendrickson *et al*., 1991). It would be interesting to evaluate *L. pulcher* against *C. asparagi* in the field in Europe to determine whether our laboratory tests are predictive of field results.

We are confident that our host specificity tests adequately demonstrate that *T. setifer, L. errabundus*, and *D. jucunda* would be restricted to *L. lilii* if released in North America. Based upon these tests, we have obtained federal and state permission for field release of all three species.

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CHAPTER 11. HOST PREFERENCE TESTING FOR PARASITOIDS OF A EUCALYPTUS BORER IN CALIFORNIA

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DESCRIPTION OF PEST INVASION AND PROBLEM

Of the more than 700 species in the genus *Eucalyptus* L'Heritier native to Australia and New Guinea, approximately 90 species have been introduced into North America over the last 150 years (Doughty, 2000). Eucalyptus trees were first propagated in California from seed brought from Australia. Insect pests and diseases associated with living trees were not introduced with the seeds. As a result, the trees growing in California were relatively free of pests until the last two decades of the twentieth century (Paine and Millar, 2002).

Phoracantha semipunctata (Fabricius) (Coleoptera: Cerambycidae) is native to Australia but has been accidentally introduced into virtually all of the *Eucalyptus*-growing regions of the world, including California, and is causing significant tree mortality in many of those areas (Paine *et al.*, 1993, 1995, 1997). The beetles are attracted to volatile chemical cues produced by downed *Eucalyptus* and *Angophora* Cav. trees, broken branches, or standing stressed trees that are suitable larval host material (Chararas, 1969; Drinkwater, 1975; Ivory, 1977; Gonzalez-Tirado, 1987; Hanks *et al.*, 1991). After mating on the bark surface, females oviposit under loose, exfoliated bark. The neonate larvae mine through the outer bark and feed in the nutritious inner bark, cambium, and outer layers of xylem (Hanks *et al.*, 1993).

Fresh host material that has a moisture content below a critical threshold (Hanks *et al.* 1991, 1999) is most suitable for larval development. Freshly cut logs attract more oviposition than aged logs (Paine *et al.*, 2001), which is consistent with observation that the adults locate available sites for oviposition by olfactory cues (Hanks *et al.*, 1991). As logs dry, the emission of volatile cues declines (Hanks *et al.*, 1998). In addition to the reduction in volatile emissions, the moisture content of the wood decreases with age and this reduces the quality of the host material for larval development (Hanks *et al.*, 1993). Larvae feeding on poor quality host material have prolonged development and smaller adult size (Hanks *et al.*, 1995). The prolonged development time exposes the larvae to increased risks of parasitism (Paine *et al.*, 2001).

TESTING RATIONALE FOR AGENTS INTRODUCED

Although *P. semipunctata* has caused significant mortality of eucalypt trees in areas of the world where it has become established, the insect has not been a significant problem in Australia (Duffy, 1963). Mortality caused by a guild of natural enemies in Australia (Austin *et al.*, 1994; Austin and Dangerfield, 1997) may be critical in regulating the population and limiting its pest status. Of the parasitoids present in Australia, the encyrtid egg parasitoid *Avetianella longoi* Siscaro and the braconid solitary larval parasitoid *Syngaster lepidus* Brullè have been introduced into California.

The Egg Parasitoid *Avetianella longoi* Siscaro

This egg parasitoid arrives on trees that are attractive to ovipositing *P. semipunctata* adults (Hanks *et al.*, 1996) and lays its eggs in beetle eggs that are less than 24 hours old (Luhring *et al.*, 2000). Since the arrival of the host beetle and parasitoid are virtually simultaneous, we expect that female wasps use cues similar to those that help adult beetles locate trees suitable for oviposition and larval development. The beetle's eggs are found in a specific microhabitat under exfoliated bark or tight crevices. Once on the host tree, female parasitoids conduct localized searches to find beetle egg masses, perhaps in response to both mechanical and odor cues. While it would have been possible to conduct formal laboratory tests with various nontarget host species found in California, in this system it is clearly the attraction of the parasitoid to the eucalypt habitat that shapes the host contacts of the parasitoid. *Eucalyptus* species are all introduced species in North America and are one of only a few groups of myrtaceous plants in California. Munz (1968) lists no members of this family as native to the state. There are very few, if any, species of herbivorous arthropods North American arthropods that have shifted onto *Eucalyptus* species as hosts. This fact strongly suggests that the risk of this parasitoid encountering alternative hosts is extremely low because of the parasitoid's strong attraction to *Eucalyptus* before searching for host eggs. Consequently, no formal host range testing was done prior to this species' release in California.

The Larval Parasitoid *Syngaster lepidus* Brullè

The larval parasitoid *S. lepidus* (Figure 1) also uses characteristics of the host tree to find *P. semipunctata* larvae in suitable stages of development. Female parasitoids are probably attracted to the plant host from a distance by volatiles emanating from the degrading/drying plant tissue. In studies conducted in Australia, levels of parasitism were significantly greater in fresh logs than logs that had been aged (Paine *et al.*, 2001). Fresh logs, even after the 10-day period for larval development, may have been a better source of those

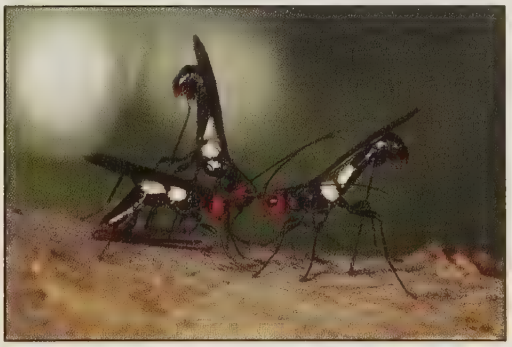

Figure 1. *Syngaster lepidus* Brullè. Photo: Don Hwan Choe. (UGA1295001)

volatiles than old logs (Hanks *et al.*, 1998). This response to volatiles produced relatively early in the process of colonization is particularly important for *S. lepidus* because females of this species prefer to use significantly smaller beetle larvae as hosts than do other species of the parasitoid guild (Paine *et al.*, 2000).

Short-range cues for location of individual larvae of subcortical beetles by parasitoid females may be a combination of sound and substrate vibration (Ryan and Rudinsky, 1962; Mills *et al.*, 1991). Once on the larval host, it is probable that female *S. lepidus* use a combination of chemical and physical cues (sonic cues or surface vibrations) to detect and evaluate the size of beetle larvae beneath the bark. *Phoracantha semipunctata* larvae feed on both the hard outer xylem tissue and the softer inner bark, producing both perceptible surface vibrations and sounds audible to the human ear for several meters. Adult wasps may perceive these vibrations through either tarsal or antennal contact with the substrate (Hanks *et al.*, 2001). Females respond to the larval stimuli and allocate the sex of their offspring directly in relation to the size of the host larvae; male eggs are oviposited on small host larvae, while female eggs are allocated to the largest larvae (Joyce *et al.*, 2002).

Host range testing of the larval parasitoid was undertaken to evaluate risk to a threatened insect, the valley elderberry longhorned beetle (*Desmocerus californicus dimorphus* Fisher). This cerambycid colonizes elderberry, *Sambucus* spp. (Caprifoliaceae), in riparian habitats in the California Central Valley. The larvae of the elderberry beetle mine the interior wood of the host plant during their 1-2 year larval life cycle.

The plant host families and the larval habitats within the host trees of *P. semipunctata* and *D. californicus* are completely different, making a comparison for host testing very difficult. Also, because *D. californicus* has a protected status, it was virtually impossible to obtain specimens for laboratory testing. Consequently, we designed a study that tested the ability of *S. lepidus* to identify and use its correct host if it was found within the plant host of the threatened insect species. We hypothesized that the parasitoid relied on host plant volatiles to locate the correct larval host habitat, and if there was a volatile stimulus from the larvae that was associated with the correct host habitat, then it would be present in both the *Sambucus* and *Eucalyptus* treatments tested in the experiment. Fresh elderberry branches were collected from the field, split open enough to expose the central pith, *P. semipuntata* larvae of a size preferred for oviposition by *S. lepidus* were placed inside, and the branches were sealed. These infested branches were placed into a bioassay cage with *Eucalyptus* logs infested with similar sized larvae and, as a control, a section of polyvinylchloride pipe. All assay items were approximately the same size. Female wasps were introduced into the assay cage and their positions were recorded at regular intervals. The results were unequivocal. They clearly demonstrated that there were no significant differences in landing and searching on either the wrong host (elderberry) or the plastic pipe. Landing on either elderberry or plastic pipe was confined to occasional periods spent resting. More importantly, the wasps spent significantly more time landing and searching in the correct host habitat than either of the other choices. There were no oviposition attempts on the *P. semipunctata* larvae in the elderberry branches, but test parasitoids did successfully oviposit on the *P. semipunctata* larvae in the *Eucalyptus* host logs.

CONCLUSIONS

Our test results indicate the important role of the host's habitat and the sequence of parasitoid behaviors that are used to locate individual hosts. The parasitoids orient initially to the stimuli associated with the habitat in which the host life stages are most likely to be found. Once within the habitat, the searching females may use different cues to locate the microenvironment of the host. In the *Eucalyptus* host plant system in North America, there are very few native plants that are closely related to eucalypts. Consequently, there are few host plants that produce similar stimuli that would attract searching parasitoids. Also, native herbivores are rarely found feeding on *Eucalyptus* plants. Since there have been no host plant shifts in more than 150 years, there are few opportunities for specialist introduced parasitoids searching the correct host to attack a nontarget native insect. While it is impossible to prove that an event will not happen, the lack of related plants, the specificity of the herbivores, and the searching behavior of the parasitoids suggest that the risk to native species is extremely low.

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CHAPTER 12. ESTIMATING THE HOST RANGE OF THE TACHINID *TRICHOPODA GIACOMELLII*, INTRODUCED INTO AUSTRALIA FOR BIOLOGICAL CONTROL OF THE GREEN VEGETABLE BUG

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BACKGROUND

Description of Pest Invasion and Problem

Nezara viridula (L.) is a cosmopolitan pest of fruit, vegetables, and field crops (Todd, 1989). The native geographic range of *N. viridula* is thought to include Ethiopia, southern Europe, and the Mediterranean region (Hokkanen, 1986; Jones, 1988). Other species in the genus occur in Africa and Asia (Freeman, 1940). First recorded in Australia in 1916, *N. viridula* soon became a widespread and serious pest of most legume crops, curcubits, potatoes, tomatoes, passion fruit, sorghum, sunflower, tobacco, maize, crucifers, spinach, grapes, citrus, rice, and macadamia nuts (Hely *et al.*, 1982; Waterhouse and Norris, 1987). In northern Victoria, central New South Wales, and southern Queensland, *N. viridula* is a serious pest of soybeans and pecans (Clarke, 1992; Coombs, 2000). Immature and adult bugs feed on vegetative buds, developing and mature fruits, and seeds, causing reductions in crop quality and yield. The pest status of *N. viridula* in Australia is assumed to be partly due to the absence of parasitoids of the nymphs and adults. No native Australian tachinids have been found to parasitize *N viridula* effectively, although occasional oviposition and development of some species may occur (Cantrell, 1984; Coombs and Khan, 1997).

Previous introductions of biological control agents to Australia for control of *N. viridula* include *Trichopoda pennipes* (Fabricius) and *Trichopoda pilipes* (Fabricius) (Diptera: Tachinidae), which are important parasitoids of *N. viridula* in the southern United States (Jones, 1988). Neither species established in Australia (Waterhouse and Norris, 1987) and would not now be considered for introduction because of their apparent lack of host specificity. Both species have a broad host range that reportedly includes species of Coreidae, Scutelleridae, Largidae, Mantidae, and Acrididiae (Arnaud, 1978; Follett *et al.*, 1999). An additional tachinid, *Bogosia antinorii* Rondani, which is native to Kenya, was introduced but similarly failed to establish (Waterhouse and Sands, 2001). Several species of parasitoids of eggs (primarily Scelionidae) have been released, of which *Trissolcus basalis* (Wollaston) has contributed to the control of *N. viridula* in southeastern Australia (Waterhouse and Norris, 1987; Waterhouse and Sands, 2001). In certain regions of eastern Australia, particularly those that produce soybeans and nut crops, *N. viridula* has remained a significant pest (Clarke and Walter, 1993; Coombs 2000).

In South America, *Trichopoda giacomellii* (Blanchard) has been shown to regulate populations of *N. viridula* in soybeans in conjunction with *T. basalis* (Liljesthrom and Bernstein, 1990; Ferreira *et al.*, 1991). Based on its performance in Argentina, *T. giacomellii* was identified as a promising potential agent for biological control of *N. viridula* in Australia (Figure 1).

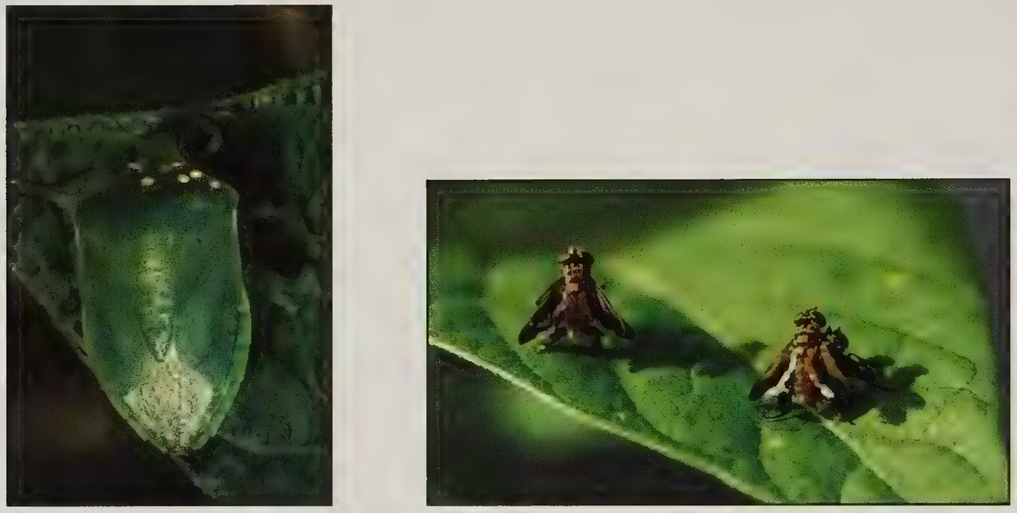

Figure 1. Adult *Nezara viridula* (L.) with parasitoid eggs attached (left) and mating pairs of *Trichopoda giacomellii* (Blanchard) (right). Photos: CSIRO. (UGA1295004 and UGA1295005)

Description of Agent Proposed for Introduction

Biology and life-history of agent Adults of *T. giacomellii* live from 4 to 15 days, and each female lays up to 275 eggs, which are deposited externally on the host thorax and abdomen (Coombs, 1997),. Supernumerary parasitism is common (2-13 parasitoid eggs per host), although only one parasitoid completes development (La Porta, 1990). Pupariation occurs in nearby soil (La Porta, 1987). Death of the host is coincident with or occurs shortly after parasitoid emergence (Coombs and Khan, 1998). Field studies in the fly's native range indicate that 80 to 90% of host individuals may be parasitized, dependent on crop type and time of year (La Porta, 1990).

Hosts in the native range of agent In Argentina, *T. giacomellii* is an important, relatively specific parasitoid of adult and late-instar nymphs of *N. viridula* (La Porta, 1990; Liljesthrom, 1991). Indigenous hosts of *T. giacomellii* in Argentina include *Acrosternum musiva* (Bergroth), *Acrosternum herbida* (Stål), *Acledra kinbergii* (Stål), *Edesia meditabunda* (Fabricius), and *Piezodorous guildinii* (Westwood) (Liljesthrom, 1980; La Porta, 1987), whereas parasitism of

N. viridula represents a new association (*sensu* Hokkanen and Pimentel, 1984, 1989). *Nezara viridula* was first reported in Argentina in 1919 (La Porta and de Crouzel, 1984). Clearly, *T. giacomellii* has a demonstrated ability to expand its host range.

Source of agent Live specimens of *T. giacomellii* were imported to Australia from La Plata, Argentina (34° 58' S, 57° 53' W) in February 1994 (Sands and Coombs, 1999). Climate matching (Sutherst and Maywald, 1985) indicated the suitability of eastern mainland Australia for survival of *T. giacomellii* (D. P. A. Sands, unpublished data).

Other known hosts. A parallel biological control program for N. viridula in South Africa, using *T. giacomellii* originating from this project, reported inclusion of the South African native pentatomids *Bathycoelia natalicola* Schouteden and *Nezara pallidoconspersa* Stål as field hosts (M. van den Berg, pers. comm.). Both species are pests of macadamia, and any potential population suppression by *T. giacomellii* was viewed as desirable.

DESCRIPTION OF FAUNA IN AREA OF INTRODUCTION

Australian Species Related to the Target Pest

No native insects in the genus* Nezara *occur in Australia Gross (1976) included *Glaucias amyoti* (White), *Alciphron glaucus* (Fabricius), and Plautia affinis Dallas, along with *N. viridula*, in a Pentatoma species group. Subsequent revision by Cassis and Gross (2002) placed *G. amyoti*, *G. sulcata*, and *A. glaucus* in the Nezarini, but *P. affinis* was transferred to the Antestini, which includes Anaxilaus (3 spp.), Antestiopsis (2 spp.), Novatilla (2 spp.), and Plautia (3 spp.). Compilation of the host test list pre-dated the Cassis and Gross (2002) revision and was based solely on the Gross (1976) species groupings. Additional species of Plautia and Glaucias and possibly other Antestini would likely have been included on the test list had the review of Cassis and Gross (2002) been available earlier.

More generally, the Australian subfamily Pentatominae contains 321 species in 113 genera, of which 303 species and 83 genera are endemic to Australia. In the family Pentatomidae as a whole, the Australian fauna includes 360 species in 135 genera, of which 330 species and 94 genera are endemic to Australia.

Local Species of Value as Biological Control Agents

Members of the predatory Asopinae (8 genera and 11 species) are of value as native biological control agents. *Oechalia schellenbergii* (Guèrin-Mèneville) and *Cermatulus nasalis* (Westwood) are common in agricultural habitats co-occurring with *N. viridula* and *P. affinis.*

Local Species of Marked Conservation Value

No Australian pentatomids are currently recognized as endangered, nor do any have icon status (Clarke and Spier-Ashcroft, 2003). Given the high degree of endemicity of the fauna (70% of the genera and 90% of the species) most Australia pentatomids represent evolutionarily unique and valuable species or groups of species.

THE TESTING PLAN: ANALYSIS OF METHODS

Selection of Species for the Test List

Species selected The list of species tested is given in Table 1.

Of the three species closely allied to *N. viridula*, *P. affinis* is a pest of agricultural and horticultural crops often found in close association with *N. viridula*, *G. amyoti* is a forest-adapted species with occasional records as a minor pest of horticultural crops; and *A. glaucus* is confined to rainforest habitats in coastal eastern Australia. All three species are native to Australia. Representative species from the families Scutelleridae, Tessaratomidae, and Coreidae were included in the host test list because the related tachinids *T. pennipes* and *T. pilipes* have some hosts in these groups. *Trichopoda giacomellii* is not known to attack these groups in its native range.

Table 1. Homoptera selected for host specificity studies with *Trichopoda giacomellii.*

Pentatomidae	
Glaucias amyoti (White)	Close relative of *N. viridula*
Plautia affinis (Dallas)	Close relative of *N. viridula*
Alciphron glaucus (Fabricius)	Close relative of *N. viridula*
Biprorulus bibax Breddin	Pest species
Piezodorous hybneri (Gmelin)	Pest species
Cuspicona simplex Walker	Pest species
Cuspicona forticornis Breddin	Locally available, added for good measure
Anaxarchus pardalinus (Stål)	Locally available, added for good measure
Oechalia schellenbergii (Guèrin-Mèneville)	Beneficial predator, agric. importance
Cermatulus nasalis (Westwood)	Beneficial predator, agric. importance
Scutelleridae	
Lampromicra senator (Fabricius)	Pest species
Tectocoris diopthalmus (Thunberg)	Pest species
Tessaratomidae	
Musgraveia sulciventris (Stål)	Pest species
Coreidae	
Amblypelta nitida Stål	Pest species
A. lutescens lutescens (Distant)	Pest species

Species that could not be tested Little or no information is available for most Australian pentatomids other than the collection records associated with physical specimens held in museum collections. Though desirable in principle, testing other poorly known genera or tribes of Australian pentatomids was impractical because of difficulty in locating such species and establishing viable laboratory cultures. Practicality dictated that host test species be selected from species about which some biological information was available, often because they were associated with agriculture as pests or beneficial species. The species selected for tests with *T. giacomellii* were, for the most part, well studied species for which we could locate detailed information on geographic distribution, habitat, host plant associations, seasonality, and in some cases, rearing methods.

DESCRIPTION OF TESTS RUN AND WHY THOSE TESTS WERE CHOSEN

Host tests were conducted as sequential, paired no-choice experiments. In each test, groups of naive *T. giacomellii* adults (n = 8-10 pairs) were exposed for 2 hours to a non-target test species (n = 10-15 adults), followed by exposure for 2 hours to the target pest, *N. viridula* (n =15 adults). This process was replicated three times for each non-target/*N. viridula* comparison. Tests thus took the form of: NT, T, NT, T, NT, T; where NT = non-target species and T = target species. Testing a given non-target/target combination required 12 hours to complete. All tests were carried in daylight hours under a 14:10 (L:D) photoperiod. *Trichopoda giacomellii* adults oviposit throughout daylight hours (M. Coombs unpublished data). At the completion of each 2 hour test period, all bugs were recovered and the numbers of parasitoid eggs per bug were recorded. For test species that attracted oviposition, appropriate food was provided in mesh screened cages until parasitoid development took place or the bugs died. All host tests were carried out in large (1.0 x 1.0 x 1.4 m) mesh screened cages constructed from aluminium frames fitted with fine cotton gauze. *Trichopoda giacomellii* adults that emerged from non-target hosts were held to record fecundity and longevity by exposing them to *N. viridula* adults in gauze cages measuring 30 x 30 x 30 cm (Sands and Coombs, 1999). Cage construction (size and material colour) did not influence parasitoid oviposition behavior.

No-choice tests were used because in choice experiments oviposition behavior of *T. giacomellii* triggered by the presence of the target host might have resulted in inadvertent oviposition on otherwise non-acceptable hosts (i.e., a false positive due to priming). This observation, however, was not tested experimentally. No-choice tests determine physiological acceptance of a particular host, and in that regard negative results are very robust, given appropriate positive controls with the target pest.

TEST RESULTS AND INTERPRETATIONS

RESULTS, SETBACKS, PROBLEMS, AND THEIR SOLUTIONS

Three native pentatomid bugs, in addition to the target pest, were identified as supporting complete development of the agent. These were *P. affinis, G. amyoti,* and *A. glaucus.* All three species are closely allied to *N. viridula* (Gross, 1976) and were found in the laboratory to be of comparable attractiveness to *N. viridula* for attack by *T. giacomellii.* Other species, including predatory Asopines, either failed to attract oviposition by the parasitoid or, when oviposition occurred, parasitoid larvae failed to develop (Sands and Coombs, 1999). Oviposition, but no development, was recorded for the pentatomids *Cuspicona forticornis* Breddin and *Anaxarchus pardalinus* (Stål). In both cases, larvae of *T. giacomellii* died as first instars while attempting to penetrate the hosts' integument. Representative examples of host test results are shown in Table 2. Approval for release of *T. giacomellii* was granted, acknowledging that some attack and development on native pentatomids might occur in the field. It was deemed that, if these nontarget hosts were encountered by *T. giacomellii* in the field, any impacts would be minor. Furthermore, any potential non-target impacts would be significantly less important than damage to crops caused by failure to control the target organism throughout Australia.

Table 2. Representative examples of sequential no-choice host tests to determine the specificity of *Trichopoda giacomellii*. Test results are presented for comparisons of *Cermatulus nasalis/Nezara viridula* and *Glaucias amyoti/Nezara viridula* exposed to *T. giacomellii* in alternating 2-hour time periods.

	Hours, Eastern Standard Time					
	0630-0830	**0831-1030**	**1031-1230**	**1231-1430**	**1431-1630**	**1631-1830**
Test species	*C. nasalis*	*N. viridula*	*C. nasalis*	*N. viridula*	*C. nasalis*	*N. viridula*
	(n =15)	(n=15)	(n=15)	(n =15)	(n=15)	(n=15)
Ave number of parasite eggs per host	0	2.2 ± 0.9	0	2.6 ± 1.4	0	2.3 ± 1.2
	0645-0845	**0846-1045**	**1046-1245**	**1246-1445**	**1446-1645**	**1646-1845**
Test species	*G. amyoti*	*N. viridula*	*G. amyoti*	*N. viridula*	*G. amyoti*	*N. viridula*
	(n =10)	(n=15)	(n=10)	(n =15)	(n=10)	(n=15)
Ave number of parasite eggs per host	0.9 ± 1.2	1.7 ± 0.7	1.1 ± 0.9	1.9 ± 1.2	0.8 ± 1.1	1.9 ± 1.1

Two recurring problems were encountered throughout the study, and both related to the location and provision of nontarget species for host testing. Invariably, despite access to detailed location data, habitat, and host plant records, considerable time was spent locating and collecting sufficient numbers of individuals required to undertake tests. Even reportedly common species were difficult to find in some instances. In most cases, we were able to collect sufficient test individuals of a given species from the field. These individuals were exposed to *T. giacomellii* and subsequently discarded following tests if no parasitoid attack occurred. When parasitoid attack did occur, test individuals needed to be kept alive long enough to allow full development of the parasitoid (requiring approximately14-16 days). As most pentatomid bugs are fruit feeders, adults could be kept alive by provision of appropriate fruit for that species. Substitute foods, such as freshly sliced green apple or dried raisins, were found to be suitable as a food source for the adults of several species. When few individuals of a given species were available from remote localities, rearing procedures were needed to provide sufficient adults for testing. Egg laying and successful development of immature pentatomid bugs often required provision of species-specific food plants (the identity of which was not always known). For *G. amyoti* and *A. glaucus,* which originated from rainforest habitats in far north Queensland, this was overcome by simultaneously providing adults with a wide range of fruiting rainforest plants. Adults were allowed to self select plant species for oviposition. Newly emerged nymphs then either remained on these plants to feed or moved by themselves to other plant species as appropriate. Host plants suitable for adult egg laying and immature development were identified using this methodology for both *G. amyoti* and *A. glaucus.*

PREDICTED VERSUS REALISED FIELD HOST RANGE OF *T. GIACOMELLII*

Release and establishment studies for *T. giacomellii* in Australia were centred on a 1400-acre pecan plantation located at Moree, New South Wales (29° 29' S, 149° 53' E). Since its establishment, *T. giacomellii* has had a sustained impact on the abundance of *N. viridula,* reducing peak abundances to 15-35% of pre-establishment densities for the years 1999 to 2002 (Coombs and Sands, 2000; Coombs, 2003). Anecdotal evidence indicates that *N. viridula* numbers have declined further as of early 2004, and it is no longer regarded as a pest in the establishment area.

Nine other pentatomid and two scuttellerid species were recorded as co-occurring with *N. viridula* in the establishment area, seven species of which were included in the pre-release host test list. Two pentatomid species, in addition to *N. viridula,* were recorded as field hosts for *T. giacomellii* at Moree (Coombs, 2003). These were *P. affinis* and *G. amyoti,* both of which were predicted to be potential hosts based on the pre-release quarantine evaluation (see above). Percent parasitism of *P. affinis* ranged from 1% to 45% on the introduced weeds *Ligustrum lucidum* Aiton and *Solanum nigrum* L., respectively. *Glaucias amyoti* was recovered only from *L. lucidum,* for which parasitism averaged less than 1%. Parasitism of *N. viridula* ranged from 9% to 70% on the same two host plants. There was no evidence of parasitism by *T. giacomellii* of the other seven species of pentatomids or two scutellerids present at the release site. Thus, no unpredicted host use was detected during the study.

The other non-target species identified as a potential host (*A. glaucus*) does not occur at the establishment site, being restricted to rainforest habitats in coastal eastern Australia. In its native range, *T. giacomellii* is apparently restricted to open rangeland and is not reported to attack pentatomids in closed-forest habitats (La Porta, 1990). Thus, habitat separation may exclude *A. glaucus* from becoming a host for *T. giacomellii* in the field.

SUMMARY

The parasitoid/host system of *T. giacomellii* and *N. viridula* gave no particular problems with regard to assessing parasitoid attack. Parasitoid eggs are attached externally to the host and easily observed. In addition, parasitoid development time is relatively short (about 2 weeks), allowing non-target species to be tested and assessed relatively quickly. The use of no-choice sequential tests appeared to give unambiguous results about which species were not hosts (i.e., negative results were robust and positive controls were obtained in controls), and test results were later conclusively supported by post-release field studies. Those species predicted to be hosts also proved to be so under field conditions, although the level of attack on *P. affinis* and, in particular, *G. amyoti* was lower than expected. Laboratory results indicated that *P. affinis*, *G. amyoti*, and *N. viridula* were of equal attractiveness to *T. giacomellii* for oviposition. The tests employed made no prediction about the effects of host plant and/or habitat on parasitoid behaviour.

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CHAPTER 13. ASSESSING HOST SPECIFICITY AND FIELD RELEASE POTENTIAL OF FIRE ANT DECAPITATING FLIES (PHORIDAE: *PSEUDACTEON*)

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BACKGROUND OF SYSTEM

Fire ant populations in their South American homeland are about 1/5 to 1/10 as dense as populations in North America (Porter *et al.*, 1992; Porter *et al.*, 1997a). This intercontinental difference in fire ant densities was not explained by differences in climate, habitat, soil type, land use, plant cover, or sampling protocols (Porter *et al.*, 1997a). Escape from numerous natural enemies left behind in South America is the most apparent explanation for the intercontinental population differences. Natural enemies left behind in South America include two species of microsporidian pathogens, three species of nematodes, about 20 species of phorid decapitating flies, a eucharitid wasp, a parasitic ant, and numerous other microbes and arthropods of uncertain relationship to fire ants (Porter *et al.*, 1997a). Escape from coevolved ant communities may also have been important. Ants in Brazil and Argentina, however, do not appear to be any more abundant than those in the United States, at least as indicated by their ability to find and occupy baits (Porter *et al.*, 1997a).

Classical or self-sustaining biological control agents are currently the only potential means for achieving permanent regional control of fire ants. Poison baits can effectively control fire ants in high value areas (Drees *et al.*, 1996; Williams *et al.*, 2001), but they are too expensive for use in rangeland and are not sufficiently specific for use in natural areas. Once initiated, baits must be reapplied two to three times each year in perpetuity, or the fire ants will return – often in even higher densities because competing ants have also been eliminated. Successful use of biological control agents will not eradicate imported fire ants, but it could help shift the ecological balance in favor of native ants. If this happened, fire ant populations in the United States could be reduced to levels similar to those in South America (Porter, 1998a).

SIMILARITY TO WEED BIOLOGICAL CONTROL

Fire ant colonies are like perennial plants in at least four ways: (1) their relative immobility, (2) their longevity, (3) the two to three years required to reach reproductive maturity, and (4) the fact that resource gathering depends on non-reproducing components (sterile workers as compared to roots and leaves). Not surprisingly, biological control of fire ants is more like exotic weed biological control than standard insect biological control. First, unlike pest populations on many agricultural crops, fire ant populations do not rise and fall dramatically in a period of a few weeks (Tschinkel, 1993). Consequently, fire ant biological control agents do not need high growth rates in order to suppress fire ant populations; their populations simply need to increase gradually until they are effective. Second, fire ant biological control agents do not need to kill their host to be effective. They only need to stress their hosts so that they have difficulty competing with other ants, just as biological control agents of weeds only need to stress their hosts so the target weeds can no longer compete with other plants in the community. Biological control of agricultural pests such as aphids, in contrast, could not rely on competition from other species of aphids to be effective as these would also be crop pests. A third similarity with weed biological control is that particular biological control agents may only be effective in certain habitats, creating a control mosaic in which single agents affect anywhere from a small fraction of all infested sites to most of the landscape. This produces a situation in which control is often best obtained by introducing a small community of natural enemies that are effective under various environmental conditions. (In contrast, crop monocultures make pest control by one or a few natural enemies more likely.) A fourth similarity is that fire ants are landscape pests that affect huge contiguous areas. While chemical control and augmentative biological control are usually cost-effective only when used on limited areas of high value, classical biological control agents are most cost effective when infested areas are large.

DESCRIPTION OF PEST INVASION

The black imported fire ant, *Solenopsis richteri* Forel, was inadvertently introduced into the United States at the port of Mobile, Alabama, around 1918 (Loding, 1929). The red imported fire ant, *Solenopsis invicta* Burden, was introduced into the same port some time during the 1930s (Lennartz, 1973). The red fire ant was by far the more successful of the two invaders. It currently occupies over 300 million acres in 12 southern states from Texas to Virginia (Callcott and Collins, 1996, USDA-APHIS, 2004). Strict quarantine procedures have significantly limited the spread of this pest (Lockley and Collins, 1990), but it has nevertheless become established in California, Australia, and across much of the Caribbean (Davis *et al.*, 2001; Nattrass and Vanderwoude, 2001; Jetter *et al.*, 2002). Unless checked, this pest has the potential to occupy tropical and warm temperate regions around the globe (Morrison *et al.*, 2004).

After its introduction in Mobile, Alabama, the black imported fire ant was driven northward by competition from the red imported fire ant and currently is restricted to a small region around the tri-state border of Mississippi, Alabama and Tennessee. However, a broad band of hybridization between red and black fire ants extends from the Mississippi River through to

Atlanta, Georgia (Shoemaker *et al.*, 1996). Red and black fire ants, however, are still considered separate species because hybridization apparently does not occur in native Argentine populations (Ross and Trager, 1990). Because black and hybrid fire ants do not occupy major ports in the United States, their opportunities for further dispersal are greatly limited. Nevertheless, dispersal out of the port of Buenos Aires in Argentina still remains a possibility.

DESCRIPTION OF THE PROBLEM

The major problem with invasive fire ants is that there are so many of them. In north Florida pastures, fire ant densities average 1,800 ants per square meter in single-queen areas and 3,500 ants per square meter in multiple-queen areas; this works out to be 15-28 kg/ha or 4-8 tons of fire ants per square mile (Macom and Porter, 1996). Economic damage in the United States is estimated at nearly 6 billion dollars per year (Lard *et al.*, 2001; Pereira *et al.*, 2002), not including environmental damage. Damage from imported fire ants can be grouped into four major categories: agricultural, electrical, medical, and environmental. Imported fire ants adversely affect several important agricultural crops, including soybeans, corn, potatoes, and citrus (Adams, 1986; Adams *et al.*, 1988; Banks *et al.*, 1991; Drees *et al.*, 1992). Fire ants are also known to prey on may beneficial insects including some biological control agents (Eubanks, 2001). Imported fire ants are also a major source of electrical problems: transformers, air conditioners, traffic switch boxes, airport lights, and other electrical equipment located on the ground are all susceptible to problems caused by fire ants chewing off insulation, jamming switches, or building mounds in electrical boxes (MacKay and Vinson, 1990; Vinson and MacKay, 1990). Medical problems from stings are the third major category of problems associated with fire ants. Young children are commonly stung dozens to hundreds of times when they stand on fire ant mounds; several people die each year from fire ant stings – mostly bedridden patients in nursing homes or people who are unconscious or otherwise unable to respond to the fire ants. About 1-2% of the population are sensitive or allergic to fire ant stings (Vinson, 1997). Environmental damage is also associated with imported fire ants. High densities of fire ants displace most native ants from open habitats (Porter and Savignano, 1990; Wojcik, 1994), especially in areas disturbed by urbanization, agriculture, or grazing. Deer, mice, shore birds, quail, and lizards are among the vertebrates that can be harmed by high fire ant populations (Allen *et al.*, 1998; Williams *et al.*, 2003).

DESCRIPTION OF AGENTS RELEASED OR PROPOSED FOR INTRODUCTION

Given the broad distribution of fire ants in North America and the magnitude of their impact, biological control appears to be most likely to be obtained by release of a suite of natural enemies. The hope is that each new self-sustaining agent will increase the magnitude and breadth of the impact on fire ant populations.

Three types of organisms are being or have been evaluated for release in the United States. Two species of microsporidians are being intensively studied: *Thelohania invictae* Knell, Allen and Hazard and *Vairimorpha solenopsae* Jouvenaz and Ellis. These pathogens slowly kill fire ant colonies in the laboratory and probably also do so in the field (Briano *et al.*, 1995; Williams *et al.*, 1999). Both diseases appear to be host specific (Briano *et al.*, 2002a). An effort will be made to obtain approval for release of one or both of these diseases from quarantine for field release trials in 2005. The parasitic ant *Solenopsis daguerrei* (Santschi) has also been evaluated as a possible biological control agent for this pest (Calcaterra *et al.*, 1999). However, so far mass rearing and transfer to red imported fire ant colonies in the United States has not been achieved (Briano *et al.*, 2002b). Phorid decapitating flies of the genus *Pseudacteon* are the third group of organisms that are being evaluated for fire ant biological control. The remainder of this chapter will discuss the biology of these flies and detail the process of studying their host specificity and evaluating risks and benefits of their field release.

Three species of South American decapitating flies have been released in the United States. The first species was *Pseudacteon tricuspis* Borgmeier in Texas (Gilbert and Patrock, 2002) and Florida (Porter *et al.*, 1999). This fly attacks medium to medium-large fire ants and is especially abundant in the fall. This species (from near Campinas, Brazil) is well established in eight states. Flies released in Florida and Alabama have spread at least 50-130 km from their release sites (Porter *et al.*, 2004). A three-year study in north Florida, however, failed to detect measurable impacts on fire ant populations (Morrison and Porter, unpubl. data); effects were apparently lower than 10-30% reduction, which was the sensitivity level of this study. A second biotype of this species from northern Argentina has been released at several sites in Texas along with the first biotype, but its establishment, while likely, still needs to be confirmed by biochemical markers. Two biotypes of *Pseudacteon curvatus* Borgmeier have also been established in the United States, one on black and hybrid fire ants in Alabama and Mississippi (Graham *et al.*, 2003; Vogt and Streett, 2003) and the other on red fire ants in Florida (Vazquez and Porter, unpubl. data) and South Carolina (Davis, Pereira and Horton, unpubl. data). This fly only attacks small fire ants and is especially abundant in the late summer. Impacts of this fly have yet to be assessed, but this fly often occurs in higher densities than *P. tricuspis.* A third species of decapitating fly, *Pseudacteon litoralis* Borgmeier, has been released at two sites in north Florida (in July and September 2003). First generation flies were recovered, but establishment has not been confirmed. This fly attacks medium-large to large fire ants and is most active in the morning and late afternoon until dark. A fourth species of decapitating fly, *Pseudacteon obtusus* Borgmeier, is being held in quarantine until permits can be obtained for its field release. Several additional species of decapitating flies are currently in quarantine in Florida and Texas, where attempts are being made to culture and evaluate them for field release. There is a consensus among phorid researchers that locally diverse communities of decapitating flies will provide more effective biological control than a single species because they will attack a broader range of fire ant sizes, in more habitats, during a broader portion of the day, and during a broader portion of the year (Morrison and Gilbert 1998; Gilbert and Patrock, 2002; Mehdiabadi and Gilbert, 2002; Folgarait *et al.*, 2003).

LIFE HISTORY OF *PSEUDACTEON* FLIES

At least 20 species of *Pseudacteon* flies have been found attacking fire ants in South America (Figure 1) (Porter and Pesquero, 2001; Brown *et al.*, 2003, unpubl. data). Up to nine species of these flies have been found at a single site (Calcaterra *et al.*, unpubl.). Each species has a distinctively shaped ovipositor that is presumably used in a lock-and-key fashion to lay eggs in a particular part of its host's body. These flies appear to be common and active throughout most of the year, but different species are sometimes more active at different times of the day (Pesquero *et al.*, 1996) and during different seasons (Fowler *et al.*, 1995a; Folgarait *et al.*, 2003). Most species are broadly distributed (Borgmeier, 1969; Borgmeier and Prado, 1975) across a wide range of habitats and climates (Folgarait *et al.*, 2004).

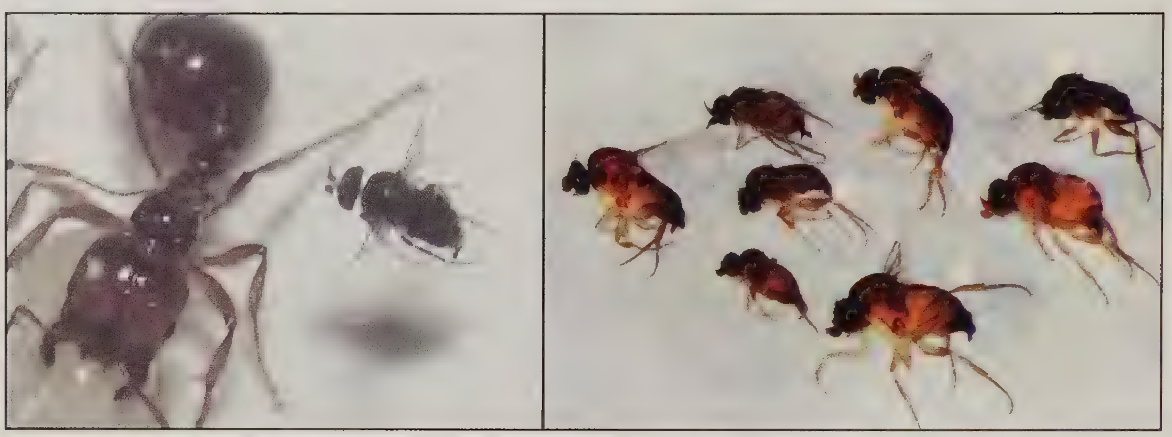

Figure 1. Female *Pseudacteon* decapitating fly preparing to attack a fire ant worker (left); eight of about 20 species of decapitating flies known to attack fire ants in South America (right). Photos: S. D. Porter. (UGA1295007)

Female *Pseudacteon* flies usually contain a hundred or more eggs (Zacaro and Porter, 2003). During oviposition, one egg is rapidly injected into the ant thorax with a short hypodermic shaped ovipositor. Shortly after hatching, maggots of *Pseudacteon* flies move into the heads of their hosts, where they develop slowly for two to three weeks (Porter *et al.*, 1995a). Just prior to pupation, the third instar maggot appears to release an enzyme that dissolves the membranes holding the exoskeleton together. The maggot then proceeds to consume the entire contents of the ant's head, a process that usually results in rapid decapitation of the living host. The headless body is usually left with its legs still twitching (Figure 2). Worker ants apparently carry the larva-infested head capsule outside their nest to above- or below-ground refuse piles several hours after the host is killed. The maggot then uses hydraulic extensions to push the ant's mouth parts aside, after which it pupates within the empty head capsule, positioned so that the anterior three segments harden to form a plate that precisely fills the ant's oral cavity (Porter, 1998a). The rest of the puparium remains unsclerotized and is protected by the ant's head capsule, which functions as a pupal case (Figure 2). Pupal development requires two to

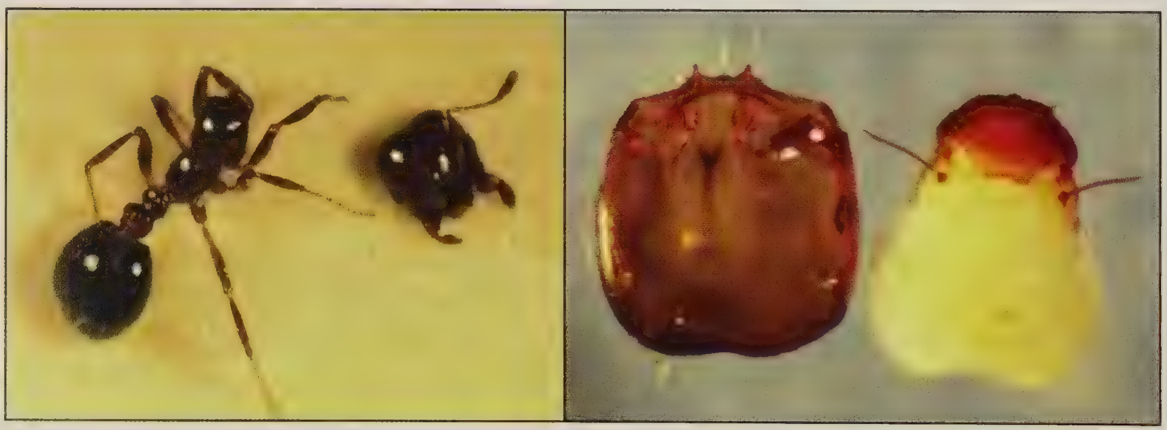

Figure 2. Fire ant worker decapitated by a *Pseudacteon* fly maggot just prior to pupariation (left); *Pseudacteon* puparium removed from fire ant head (right). Photos: S. D. Porter. (UGA1295008)

three weeks depending on temperature. Adult flies are generally mature and ready to mate and oviposit about three hours after emergence. Based on laboratory observations, adult *Pseudacteon* flies may live up to a week in nature; however, high rates of activity associated with oviposition will shorten their lives to one to three days.

During attacks, fire ant workers are keenly aware of the presence of phorid flies. A single female fly usually stops or greatly reduces the foraging efforts of hundreds of fire ant workers in only a minute or two (Porter *et al.*, 1995b). As soon as a fly appears, most workers rapidly retreat into exit holes or find cover. Other workers curl into a stereotypical c-shaped posture (Porter, 1998a; Wuellner *et al.*, 2002a), a behavior not seen except when the ants are under attack by phorids. Some fly species inhibit fire ant foraging as long as they are present, often for periods of several hours (Folgarait and Gilbert, 1999; Wuellner *et al.*, 2002b). Reduced foraging activity appears to facilitate competition from ants that might otherwise be excluded from food sources in fire ant territories (Feener, 1981; Orr *et al.*, 1995; Morrison, 1999; Mehdiabadi and Gilbert, 2002). Several flies are sufficient to stop nest construction or freeze the activity of entire colonies in laboratory nest trays (Porter *et al.*, 1995b). The overall impact of these flies on fire ant populations is unknown; however, it is clearly sufficient to have caused the evolution of a number of phorid-specific defense behaviors.

THE RECEIVING LOCATION

Description of Fauna in Area of Proposed Agent Introduction

Evaluation of potential non-target impacts generally begins with identification of potential hosts that are closely related to the target host. Black and red imported fire ants have two native fire ant congeners in the southeastern United States: the tropical fire ant, *Solenopsis geminata* (Fabricius), and the southern fire ant, *Solenopsis xyloni* (MacCook). Both ants can be pests, but they rarely reach the same high densities of their imported cousins. The invasion of imported fire ants generally results in a dramatic decline of the number of *S. geminata* colonies and the elimination of all *S. xyloni* populations (Porter, 2000). Two native desert fire ants, *Solenopsis aurea* Wheeler and *Solenopsis amblychila* Wheeler, occur in the southwestern United States. These species are much less common, and it is generally assumed that they occur in habitats too dry for direct competition with imported fire ants. A dozen or so species of thief ants also occur in the southern United States; while these ants are also in the genus *Solenopsis*, they are not potential hosts of decapitating flies because of their extremely small size.

Other genera of ants in the subfamily myrmicinae are the next closest relatives of fire ants, followed by ants in other subfamilies. However, ant head size is probably more critical than subfamily, judging by the fact that species of *Pseudacteon* flies have been found parasitizing similar-sized ants in the subfamilies Formicinae, Dolichoderinae, and Ecitoninae (Disney, 1994). Very small ants (< 0.4 mm, head width) and large ants (>1.6 mm, head width) apparently lack *Pseudacteon* parasites.

In contrast to more conspicuous insects like butterflies, no ant species are listed as rare or endangered. A number of native ants have very interesting habits to myrmecologists, but only perhaps *Pogonomyrmex* harvester ants and *Atta* leaf-cutter ants would stand out as iconic species to the public where they occur. Nevertheless, ants in general play important roles in food webs (Folgarait, 1998), and at least one threatened vertebrate, the Texas horned lizard (*Phrynosoma cornutum* (Harlan)), depends heavily on native ants, particularly harvester ants, for food (Blackshear and Richerson, 1999). It is therefore important that potential fire ant biological control agents be restricted as much as possible to imported fire ants.

Fire ants and a few other species of ants are considered beneficial to a few crops, especially sugarcane in which they help control the sugarcane borer (*Diatraea saccharalis* Fabricius). Consequently, some sugarcane growers vociferously object to biological control efforts directed at imported fire ants. However, the benefits to sugarcane production would be less clear if the comparison was between "fire ants and other ants" rather than "fire ants and no ants" (Adams *et al.*, 1981). In Texas rangelands, ticks are virtually absent where fire ants have invaded, so ranchers are likewise concerned about imported fire ant biological control (Fleetwood *et al.*, 1984). In regard to these special concerns, it is important to keep in mind that the net impacts of imported fire ants across all areas of agriculture, human health, and wildlife are overwhelmingly negative (Lard *et al.*, 2001; Wojcik *et al.*, 2001).

THE TESTING PLAN: ANALYSIS OF METHODS

Four categories of pre-release host range studies have been conducted with fire ant decapitating flies: (1) literature searches, (2) general field observations, (3) field tests in the native range, and (4) laboratory tests in quarantine. In addition, (5) a battery of food preference tests were conducted to determine if the flies were attracted to food items that might make them a nuisance or a potential disease vector. Finally, (6) several post-release tests were conducted to test the effectiveness of pre-release predictions.

LITERATURE SEARCHES

Literature searches provided important data about hosts in the field, geographic distribution, and the hosts of related species of phorids. The taxonomic publications of Thomas Borgmeier in Brazil were especially important (Borgmeier, 1921, 1925, 1962; Borgmeier and Prado, 1975).

FIELD OBSERVATIONS

Information from the literature was also supplemented by additional field observations in the past decade as we and our colleagues conducted studies of the ecology and behavior of phorids and their hosts in South America

FIELD TESTS IN THE NATIVE RANGE

Field tests in the native range were conducted with *Pseudacteon* flies to confirm data from the literature that indicated a general host specificity to fire ants. The first set of tests was conducted by setting out clusters of four to nine trays with different species of ants, one of which contained South American fire ants (Porter *et al.*, 1995c). The second set of tests was conducted by setting out tropical fire ants (*S. geminata*) in trays for 30 minutes followed by red fire ants for a similar amount of time (Porter, 1998b). These tests were particularly useful because *S. geminata* occurs in both Brazil and the United States.

LABORATORY TESTS IN QUARANTINE

The tropical fire ant (*S. geminata*) was selected as the primary native ant species to test because it was the most common native congener in the United States to imported fire ants. The theory was that a fly that would not attack a fire ant congener would also be very unlikely to attack ants in other genera. A second native fire ant, *S. xyloni*, was added for tests with *P. curvatus* after tests showed that *P. curvatus* would develop in *S. geminata*. Both *S. geminata* and *S. xyloni* were used in most subsequent tests in an attempt to be more thorough and because data collected so far indicate that *Pseudacteon* flies are more likely to parasitize *S. xyloni* than *S. geminata*. Native non-*Solenopsis* ants were also added if flies were capable of developing in either species of native fire ant. Non-native ants were selected from as many different genera as possible – giving preference to species of appropriate size for the fly being tested. Specificity tests have not been conducted with either of the desert fire ants (*S. aurea, S. amblychila*) because colonies of these species are difficult to obtain and their desert habitat would seem to make them less susceptible to *Pseudacteon* flies from South America. Furthermore, it was judged that negative impacts on the desert fire ants were unlikely to exceed those found on *S. geminata* and *S. xyloni* and that, even if they were somewhat higher, this would be unlikely to stop the release of a promising agent that would be far more likely to help protect whole communities of ants by its impacts on imported fire ants.

No-choice tests No-choice tests were the first tests conducted in the laboratory. The objective was to determine whether a particular species of fly had the motivation and capability of attacking a potential host ant when no other alternatives existed. No-choice tests were initially conducted with flies that had been hand carried up from South America and immediately tested in quarantine in the one to three days before they died (Porter, 1998a). Because the viability and motivational status of many field-caught flies were suspect, sequential tests were used. Gilbert and Morrison (1997) and Morrison and Gilbert (1999) tested individual flies with imported fire ants, then with native fire ants, and finally with imported fire ants again. Flies not showing activity against imported fire ants were not used in tests with native fire ants. This method tested the specificity of flies known to be motivated to attack the normal host, thus reducing the chances of false negatives due to stress or age. Porter and Alonso (1999) also used sequential testing. Half of their tests were done first with imported fire ants and then with native ants; the other half of the tests were done first with native ants and then with imported fire ants. This method assessed both the host specificity of flies that had been primed for attack with imported fire ants and those that had not.

Additional no-choice tests have also been conducted using flies reared from laboratory cultures in quarantine. For these tests, 15-20 flies were generally placed into attack boxes (Figure 3); several boxes usually contained test ants and the others contained imported fire ants used as controls or standards. Five to nine replicate trays were run for each species tested. The percent of hovering flies, attack rates, and parasitism rates were determined for each test box. Test flies remained in the boxes until they died of old age or died of the perils of attacking fire ants, usually 1-3 days. The advantage of using flies from laboratory cultures was that much larger numbers of flies could be tested over their full adult lifespan. The disadvantage was that 6-12 months of labor were already invested in rearing the flies before determining whether they would be suitable for field release. This risk was acceptable after we determined from the previous tests that most flies would likely be suitably host specific.

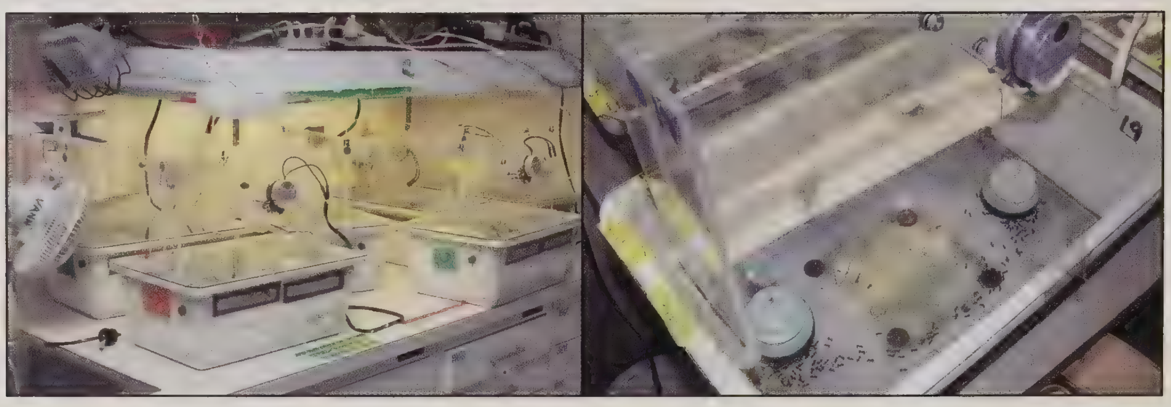

Figure 3. Attack boxes used in no-choice tests at the USDA quarantine facility in Gainesville, Florida. The electric motor on top of the box drives a cam that raises and lowers the green and black lids inside the box every 15 minutes so that ants trail back and forth while the flies attack. Photos: S. D. Porter.

Another alternative to transporting short-lived flies to the United States for specificity tests would be to transport *geminata* complex ants (*S. geminata* and *S. xyloni*) to quarantine facilities in South America, where it would be much easier collect flies for testing. This could be done safely if sterile workers without queens or sex brood were used. Conducting host range tests in South America with potential North American hosts is a tempting possibility, but this would entail resolving serious issues of trust, responsibility, and perceptions before permits could be obtained. Host range studies involving the importation of pest ant biotypes back to their country of origin, however, has been arranged for *Pseudacteon* parasitoids of Argentine ants (Orr *et al.* 2001).

Choice tests Paired choice tests were usually only conducted after no-choice tests indicated that a particular species of fly was capable of attacking and developing in a native non-target fire ant host (Porter, 2000; Vazquez *et al.*, 2004). The objective was to determine whether the flies were likely to have strong preference for imported fire ants when they occurred in micro-sympatry (i.e., when near the same resource) with native fire ants. Choice tests were also used to assess host range preferences of flies attacking fire ants within the *saevissima* complex in South America. Choice tests were never done between *Solenopsis* and non-*Solenopsis* ants because the *Pseudacteon* test species rarely attempted to attack non-*Solenopsis* ants and never successfully parasitized them. Paired choice tests were conducted in specially modified attack chambers, each of which contained two parallel trays in the bottom – one for each of the host pairs. Tests were generally run for two to three hours with 10-15 flies in each tray. The paired chambers were also used to test two non-*Solenopsis* ants at a time because it was anticipated that flies would not be motivated to attack either ant species.

FEEDING PREFERENCE TESTS

Many kinds of flies can be a nuisance or even a health hazard if they are attracted to humans, animals, fruits, prepared foods, carrion, feces, or dung. While not directly related to host range, it was important to determine whether *Pseudacteon* flies were likely to vector diseases or become a nuisance problem. To investigate this potential, unfed flies were placed into trays with a smorgasbord of potential food items (Porter, 2000). Chi-square tests were used to see if they were attracted to any of the potential food items more frequently than they were to moist tissue paper.

POST-RELEASE SPECIFICITY TESTS

Post-release specificity tests were conducted in the field in order to determine whether pre-release assessments of host specificity were accurate predictions of what happened in the field (Morrison and Porter, 2005; Vazquez and Porter, 2005). These tests were conducted in two ways. The first was to look for flies attracted to disturbed native and imported fire ant mounds. The second was to place trays of ants in the field, as was done in South America. In tests with trays, native fire ants or native ants from other genera were put out in trays for 20-30 minutes, after which they were replaced with trays of imported fire ants to confirm the presence of the flies. Then the imported fire ants were removed and the native ants were replaced to see if they would be attacked by motivated flies after they had been attracted to the trays.

TEST RESULTS AND INTERPRETATIONS

LITERATURE SEARCHES

In the literature, ants were listed as the presumptive host for all known flies in the genus *Pseudacteon* (Wasmann, 1918; Borgmeier, 1921, 1925, 1969; Borgmeier and Prado, 1975; Disney, 1994). Furthermore, virtually all phylogenetically related phorid genera are also apparently ant parasites (Brown, 1993; Disney, 1994). The life cycle of *Pseudacteon* flies (Porter *et al.*, 1995a) strongly suggests a high degree of host specificity. In particular, the puparium is highly modified to fit snugly in the head capsule of a decapitated ant. This information suggests that only ants and probably only ants of a particular size range would be suitable hosts. Similarly, the well developed and distinctive ovipositors also suggest a high degree of host specificity.

The literature further indicated that almost all *Pseudacteon* species are only attracted to worker ants in a single genus. One species (*P. borgmeieri* Schmitz) was reported to attack both *Solenopsis* and *Camponotus* ants, but on investigation, this turned out to be a translational error (Porter *et al.*, 1995c). Another rare species (*Pseudacteon convexicauda* Borgmeieri) has been collected hovering over both *Solenopsis* and *Paratrechina* ants, but field observations suggest that it as actually a parasite of *Paratrechina* workers (Porter and Pesquero, 2001). Perhaps it is occasionally collected over fire ants when *Paratrechina* ants are mixed in. *Pseudacteon formicarum* (Verrall) in Europe has been reported hovering over several genera of ants, but Wasmann argued it was only a parasite of *Lasius* ants (Wasmann, 1918). Ultimately, rearing tests will be necessary in order to resolve questions about this species and the previous one.

Finally, the literature indicated that *saevissima* and *geminata* complex fire ants have distinct communities of *Pseudacteon* parasites. At least four species of flies are known to parasitize *S. geminata* and/or *S. xyloni* in the United States, but they were never collected attacking imported fire ants (both in the *saevissima* complex) in the United States, even though they clearly would have had the opportunity to do so (Smith, 1928; Morrison *et al.*, 1999b). Similarly, *Pseudacteon* parasitoids of *saevissima* complex fire ants have not been reported attacking *geminata* complex fire ants even though there is broad geographic overlap between these two groups in northern South America. Two species of flies, *Pseudacteon solenopsidis* (Schmitz) and *Pseudacteon wasmanni* (Schmitz), were reported in the literature as attacking fire ants in both the *geminata* complex and the *saevissima* complex (Disney, 1994), but the reports for *geminata* turned out to be an early misidentification (Schmitz, 1914) of what was almost surely *saevissima* complex ants (Fowler *et al.*, 1995b).

In the Americas, *Pseudacteon* phorids can be typified as fire ant specialists because most species are fire ant parasitoids (Disney, 1994). The observation that *Pseudacteon* flies have only colonized a few non-*Solenopsis* ant genera over evolutionary time, and the fact that North American *Pseudacteon* flies using *geminata* complex ants have failed to colonize either red or black imported fire ants in 7-8 decades of exposure is testimony to the powerful constraints against switching hosts. These constraints appear to relate to the use of host pheromones in locating workers to parasitize (Morrison and King, 2004). Moreover, the fact that host ants are under pressure to evolve unique chemical signals may account for the species-level specialization we often observe in this system.

FIELD OBSERVATIONS

With regard to field observations taken in the course of behavioral and ecological studies, the Gilbert and the Porter research groups and their various colleagues in Brazil and Argentina began intensive field observations in the early 1990s. The host specificity patterns apparent in the literature were confirmed by hundreds of hours of field observations taken over baits where *saevissima* complex ants interacted with many other ant genera ($n > 20$). In summary, information in the literature, supplemented by extensive field observations, indicate that *Pseudacteon* flies that attack fire ants would be specific to fire ants. Furthermore, it is also likely that flies that attack imported fire ants would prefer imported fire ants over native fire ants.

FIELD TESTS IN THE NATIVE RANGE

The host specificity of *Pseudacteon* flies was initially tested in the field at three locations in South America with 23 species of ants from 13 genera (Porter *et al.*, 1995c). *Pseudacteon* flies, primarily *P. litoralis* and *P. wasmanni,* but also lower numbers of *P. tricuspis, Pseudacteon pradei* Borgmeier, *P. curvatus,* and *P. borgmeieri,* were attracted only to *Solenopsis* fire ants. Three individuals of two species of flies (*P. wasmanni* - 2, *P. pradei* - 1), however, were attracted to a tray containing black *S. geminata* fire ants.

A second set of field tests was conducted with three colonies of black *S. geminata* and three colonies of *saevissima* complex ants. Trays with these colonies were set out two times at each of two sites near Rio Claro, Brazil (Porter, 1998b). When the *S. geminata* colonies were set out, they initially attracted no flies; however, when the *saevissima* complex ants were set out, flies were always attracted to each tray with *saevissima* ants (12 of 12 opportunities). When all the trays were placed together at one location, flies were again attracted to all of the *saevissima* trays, but only one *S. geminata* tray briefly had a *P. litoralis* fly that hovered but did not attempt to oviposit. When the *saevissima* trays were removed, leaving only *S. geminata* trays, a total of five flies hovered over a *S. geminata* tray on four of 12 occasions. One fly (*P. wasmanni*) was observed systematically attacking *S. geminata* workers. When the *saevissima* trays were returned, all of the flies selected ants in the *saevissima* trays. At the end of the experiment, 588 fly larvae were reared from the three *saevissima* trays, compared to 12 larvae from the *S. geminata* trays. The 262 flies that emerged from the *saevissima* trays were 52% *P. tricuspis*, 39% *P. litoralis*, 5% *P. wasmanni*, *P. pradei* 3%, and 0.4% *P. curvatus*. No flies emerged from the *S. geminata* trays, but at least three of the pupae from these trays were *P. wasmanni*.

The results of these field tests in Brazil showed that the *Pseudacteon* species that attack fire ants were not attracted to ants in other genera. Tests with *S. geminata* showed that *P. litoralis* and *P. tricuspis* largely ignored *S. geminata* workers. However, *P. pradei* and *P. wasmanni* would hover over *S. geminata* workers and *P. wasmanni* was probably capable of completing development in *S. geminata*. Nevertheless, when both *saevissima* complex workers and *S. geminata* complex workers were present, all of the *Pseudacteon* flies at the test site selected *saevissima* complex workers.

LABORATORY HOST RANGE TESTS

No-choice tests with native congeners Laboratory no-choice tests in quarantine facilities show that *P. tricuspis* and *P. litoralis* have a high degree of host specificity for the red imported fire ant *S. invicta* over the native fire ants *S. geminata* and *S. xyloni* (Table 1). Females of *P. litoralis* and *P. tricuspis* occasionally hovered over native fire ant workers but only at 0-15% of the rates that they did over *S. invicta* workers. Oviposition attempts were even rarer. *Pseudacteon litoralis* females have never successfully parasitized *S. invicta* workers and *P. tricuspis* females have only succeeded once when dead *S. invicta* workers were mixed in with live *S. geminata* workers. *Pseudacteon wasmanni* flies also showed high specificity to the red imported fire ant when compared to *S. geminata*, but more tests will be necessary because test numbers were low (Table 1) and the field trials indicated the potential for this species to develop in native fire ants (Porter, 1998b). Preliminary tests also indicated that *Pseudacteon nudicornis* Borgmeier is highly host specific. The small unnamed species near *P. obtusus* from Campinas, Brazil, was also highly host specific to imported fire ants. *Pseudacteon obtusus* flies from Herradura and Corrientes, Argentina were able to attack small numbers of native fire ants and development was confirmed in the Herradura flies (Table 1). *Pseudacteon curvatus, P. borgmeieri,* and *Pseudacteon nocens* Borgmeier were the least host specific of the flies tested as far as their attack rates (Table 1). *Pseudacteon curvatus* females hovered over native fire ants at about 2/3 of the rate over the red imported fire ant, and parasitism rates ranged from 0-35% of the rate for *S. invicta* depending on the host and the origin of the fly. Several *P. borgmeieri and P. nocens* flies also readily attacked native *S. geminata* fire ants (Table 1), but no data are available about whether they are able to successfully parasitize them. While 36% of *P. nocens* females attacked *S. geminata*, they did so at 1/6th the rate with *S. invicta* (Gilbert et al., unpubl. data). *Pseudacteon cultellatus* Borgmeier attacked *S. geminata* in low numbers (Table 1), but sample sizes are still too low to be precise and it is not known whether they can parasitize native fire ants.

Choice tests with native congeners Paired choice tests were run with *P. curvatus* from Las Flores and Formosa (both locations in Argentina) and *P. obtusus* from Herradura, Argentina. These tests were undertaken because both species had the ability to attack and develop in native fire ants; therefore, it was important to know whether these flies had a preference for

Table 1. Percentage rates of attack or parasitism of native fire ants (*S. geminata, S. xyloni*) compared to rates for the target host the red imported fire ant (*S. invicta*) for ten species of South American *Pseudacteon* flies.

Fly Parasitoid Species Source (# tested)	**Attack Behavior**		**Parasitized Workers**		**Literature Source**
	S. geminata	***S. xyloni***	***S. geminata***	***S. xyloni***	
	(% of rate on *S. invicta*)		**(% of # on *S. invicta*)**		
Pseudacteon litoralis					
Campinas, BR (23)	9	—	0	—	Gilbert & Morrison, 1997[a]
Jaguariúna, BR (68, 51)	7	—	0	—	Porter & Alonso, 1999[b]
San Justo, AR (20, 15)	0	3	0	0	Porter, unpublished[c]
Pseudacteon tricuspis					
Campinas, BR (25)	4	—	0	—	Gilbert & Morrison, 1997[a]
Jaguariúna, BR (84, 72)	5	—	0	—	Porter & Alonso, 1999[b]
Formosa, AR (27, 27)	8	15	0	0	Porter, unpublished[c]
Pseudacteon wasmanni					
Campinas, BR (18)	11	—	0	—	Gilbert & Morrison, 1997
Jaguariúna, BR (9)	0	—	0	—	Porter & Alonso, 1999[b]
Pseudacteon nudicornis					
Santiago del Estero, AR (6)	0	—	—	—	Gilbert, *et al.*, unpublished[a]
Pseudacteon* sp. near *obtusus					
Campinas, BR (18)	0	—	—	—	Morrison & Gilbert, 1999[a]
Pseudacteon obtusus					
Herradura, AR (102, 102)	14	29	4	13	Porter, unpublished[c]
Corrientes, AR (8)	13	—	—	—	Gilbert, *et al.*, unpublished[a]
Pseudacteon curvatus					
Campinas, BR	65	—	12	—	Gilbert & Morrison, 1997[a]
Las Flores, AR (180, 140)	11	71	6	35	Porter, 2000[c]
Formosa, AR (150, 130)	64	77	0	13	Vazquez *et al.*, 2004[c]
Pseudacteon borgmeieri					
Jundiai, BR (3)	67	—	—	—	Morrison & Gilbert, 1999[a]
Buenos Aires, AR (2)	100	—	—	—	Morrison & Gilbert, 1999[a]
Pseudacteon nocens					
Santiago del Estero, AR (61)	36	—	—	—	Gilbert, *et al.*, unpublished[a]
Pseudacteon cultellatus					
Santiago del Estero, AR (12)	8	—	—	—	Gilbert, *et al.* unpublished[a]

[a] Attack behavior data are calculated from the percentage of females that also attacked *S. geminata* after they had attacked *S. invicta*.

[b] Attack behavior data are calculated from the total number of flies attacking *S. geminata* as a percent of the number that attacked *S. invicta*.

[c] Attack behavior data are calculated from the mean number of flies hovering in attack mode during the observation period in *S. geminata* or *S. xyloni* boxes as a percent of the mean number observed in the *S. invicta* boxes.

imported or native fire ants when they co-occurred. For *P. curvatus*, the results were that 75-85% of the female flies preferred the imported fire ant over either native fire ant (Porter, 2000; Vazquez *et al.*, 2004). Females reared on *S. xyloni* retained a strong preference for *S. invicta*, indicating that host preferences are genetically hardwired rather than facultatively determined by rearing history (Porter, 2000). About 95% of *P. obtusus* females chose to attack *S. invicta* over either native species (Porter, unpublished data).

Host range tests with* saevissima *complex fire ants In addition to studying host range to determine impacts on non-target organisms, it is also important to determine whether the biological control agents being studied can successfully attack the target hosts. Adequate host range breadth to attack all target species is important because both the red fire ant, *S. invicta,* and the black fire ant, *S. richteri,* occur in the United States. Field collection data in South America showed that most species of decapitating flies were broadly distributed across the ranges of several species of fire ants such that they must use several different species as hosts (Borgmeier and Prado, 1975; Porter and Pesquero, 2001; Folgarait *et al.*, 2004). Additional studies have demonstrated that flies from a specific location are usually capable of attacking and parasitizing several species of fire ants in the *saevissima* complex (Porter and Briano, 2000; Folgarait *et al.*, 2002a; Folgarait *et al.*, 2002b). Nevertheless, early tests with *P. tricuspis* from Argentina suggested that flies collected from black fire ants preferred black fire ants (Porter *et al.*, 1997b). Subsequent laboratory tests have confirmed that *P. tricuspis* flies from red fire ants prefer red fire ants and *P. tricuspis* flies from black fire ants prefer black fire ants, although flies were capable of parasitizing either host (Porter, unpubl. data). Laboratory tests with *P. curvatus* collected from red and black fire ants showed the same pattern (Porter and Briano, 2000, Vazquez and Porter, unpublished data).

No-choice tests with ants in other genera No-choice tests have been conducted in quarantine with five species of flies (Table 2). About 2% of *P. litoralis* females (1/51), 5% of the *P. tricuspis* females (3/61), and none of the *P. wasmanni* females (0/6) hovered over ants in another genus and appeared to attempt oviposition (Porter and Alonso, 1999). The *P. curvatus* flies hovered over the native ants at about 14% of the rate that they did over *S. invicta* workers. In most cases, a few oviposition attempts were also observed (Porter, 2000). None of the *P. obtusus* flies hovered over any of the non-*Solenopsis* ants in the quarantine tests (Porter, unpubl. data).

All ant species in which oviposition attempts were seen were maintained for 4-5 weeks and observed for signs of parasitism. Two species of native phorid parasites were found; however, none of the test ants in other genera were parasitized by any of the five species of South American decapitating flies being tested.

FEEDING PREFERENCE TESTS

A thorough review of the literature showed no reports of *Pseudacteon* flies being attracted to fruit, animals, prepared food, carrion, feces, or dung in South America. None of the decapitating flies were ever attracted to the authors, their colleagues, or their lunches during many hours of collecting activities in the field (Porter, unpubl. data; Porter, 2000). The flies *P. litoralis* and *P. tricuspis* were not attracted to prepared foods, sugar solutions, oils, fruits, feces, or flowers during rearing tests in Rio Claro, Brazil (Porter, unpubl. data). *Pseudacteon tricuspis* flies from Buenos Aires, Argentina were also not attracted to various types of foods presented during rearing tests although they were observed to lap up sugar and honey water if these were encountered (Porter *et al.*, 1997b). *Pseudacteon curvatus* flies were tested with more than 50 potential food items, including fruits, vegetables, raw meat, prepared foods, carrion, and dung. The flies showed no more attraction to any of the test items than they did moist tissue paper balls used as controls; in fact, 75% of the flies never visited any of the test items (Porter, 2000).

Table 2. Genera of non-*Solenopsis* ants used in no-choice tests with phorid decapitating flies in quarantine; none of these ants were successfully parasitized.

***Pseudacteon* Fly Species**	**Non-Solenopsis Ant Genera (number of species) Tested**
Pseudacteon litoralis[a]	*Aphaenogaster* (2), *Camponotus, Crematogaster, Neivamyrmex, Pheidole*
Pseudacteon tricuspis[a]	*Aphaenogaster* (2), *Camponotus, Crematogaster, Forelius, Pheidole*
Pseudacteon wasmanni[a]	*Aphaenogaster. Crematogaster*
Pseudacteon curvatus[b]	*Aphaenogaster, Camponotus, Crematogaster* (3), *Dorymyrmex* (2), *Forelius, Lasius, Leptothorax, Linepithema, Pheidole* (5), *Pseudomyrmex, Tetramorium, Trachymyrmex*
Pseudacteon obtusus[c]	*Aphaenogaster* (2), *Camponotus* (2), *Crematogaster, Cyphomyrmex, Dorymyrmex, Formica, Odontomachus, Pheidole* (2), *Pseudomyrmex, Tetramorium, Trachymyrmex*

[a]Porter and Alonso, 1999; [b]Porter, 2000 (Las Flores biotype); [c]Porter, unpublished data

POST-RELEASE FIELD TESTS

Specificity tests conducted in the field about three years after *P. tricuspis* had been released in north Florida showed no signs of non-target effects (Morrison and Porter, 2005). Flies were not attracted to mounds of the native fire ant *S. geminata*. They were also not attracted to trays with native fire ants or to trays with 14 other species of ants in 12 different genera. These results were congruent with what had been predicted by laboratory and field host specificity tests (Gilbert and Morrison, 1997; Porter, 1998a; Porter and Alonso, 1999).

No-choice post-release specificity tests were also run with the Formosa biotype of *P. curvatus* about 8 months after field release (Vazquez and Porter, 2005). A few flies were attracted to trays with *S. geminata* ants, but the rates of attraction were less than 5% of those observed with the red imported fire ant, and the few flies that came generally hovered for a few minutes without attacking and then left. These results were much better than predicted by laboratory tests in which the hovering rate over *S. geminata* was about 66% of the rate for *S. invicta* for no-choice tests and about 15% for choice tests (Vazquez *et al.*, 2004). The native fire ants were not checked for parasitism because only one possible attack was observed and the flies had never successfully parasitized *S. geminata* fire ants in the laboratory. No *P. curvatus* flies were attracted to any of the 15 other species of ants in 12 other genera that were offered in test trays.

Post-release specificity tests have also been done with the Las Flores biotype of *P. curvatus* in Alabama, where they are established on hybrid (red x black) fire ants from a field release about three years earlier (Graham *et al.*, 2003). Some females were attracted to trays with the native fire ant *S. geminata* and attacks were commonly observed; however, the rate of parasitism in *S. geminata* workers was about 3% of the rates with *S. invicta* or hybrid fire ants (Porter and Graham, unpubl. data). This is a little less than would have been predicted by the laboratory tests (Porter, 2000). Tests with ants in other genera still need to be done.

RISK ASSESSMENTS

Pseudacteon tricuspis* and *Pseudacteon litoralis The risk to native fire ants from the *P. tricuspis* and *P. litoralis* is very small. Field tests show that these species are not attracted to *S. geminata* fire ants (Porter, 1998b). Even under forced laboratory conditions, almost all female flies refused to attack *S. geminata* workers (Gilbert and Morrison, 1997; Porter and Alonso, 1999). However, *P. tricuspis* will attack *S. geminata* under unusual circumstances and did, in one instance, complete development in an *S. geminata* worker (Porter and Alonso, 1999). A small risk to *S. geminata* is acceptable for four reasons. First, this species, while being native to the United States and other parts of the Americas, is a pan-tropical pest (Trager, 1991). In fact, it would probably be ranked as one of the most important exotic ant pests in the world (Williams, 1994). Fortunately for us, its densities here have never approached those of the imported fire ants (Porter, 1992). Secondly, this ant and its sister species, *S. xyloni,* already have at least four species of *Pseudacteon* phorids that attack them in the United States but that do not attack the imported fire ants (Morrison *et al.*, 1997). Consequently, it seems very unlikely that imported *Pseudacteon* species that are not even attracted to *S. geminata* could switch to a new host and out-compete the phorid parasites that have already coevolved with it. Thirdly, the range of *saevissima* complex fire ants in South America overlaps broadly with that of *S. geminata* (Trager, 1991); thus, most of the phorid parasites of *saevissima* complex ants have probably already had millions of years to make the jump to *S. geminata*, but without success. Fourthly, *S. invicta* is slowly displacing *S. geminata* from most of its range in the United States (Porter, 1992; Wojcik, 1994): in other words, the clear and present danger that *S. invicta* posses to *S. geminata* is much greater than the small risk that introduced *Pseudacteon* flies would have. This final argument is also applicable to *S. xyloni* because the imported fire ant *S. invicta* has totally eradicated *S. xyloni* from almost all of its former range in the southeastern United States. Consequently, we can wait and permit *S. invicta* to continue eradicating *S. xyloni*, or we can take a small risk with importing several parasitic flies that may help reverse this trend—especially as *S. xyloni* is a pest species in its own right (Smith, 1965).

Pseudacteon curvatus The laboratory and field host range information indicate that release of *P. curvatus* may pose a small risk to native fire ants (Porter, 2000). Release of this fly, however, is much more likely to benefit native fire ants because imported fire ants are their principal competitors, and these flies will almost certainly have much a greater effect on imported fire ants than on native fire ants. In short, risks to native fire ants need to be balanced against potential benefits to native ants in other genera and numerous other native organisms that are negatively affected by imported fire ants, including numerous rare and endangered species. Nevertheless, release of this species in regions such as Texas where native fire ants and their phorid faunas persist in some local areas behind the invasion front was delayed until we gained a better perspective on how *P. curvatus* might affect the system and could judge the prospects of developing comparable "small phorid" alternatives quickly (Gilbert and Patrock, 2002).

Much of the prior discussion concerning risk for *P. tricuspis* and *P. litoralis* also applies to *P. curvatus,* but several additional considerations are also important in regard to the field release of *P. curvatus*: (1) *P. curvatus* and other *Pseudacteon* species will, at best, stress imported fire ant populations, thus reducing their ability to compete with native ants. Consequently, there is no chance that releasing *P. curvatus* will eradicate *S. invicta* or any of the native fire ants. (2) *P. curvatus* is among the smallest species of *Pseudacteon* flies that attack *S. invicta* (Morrison *et al.*, 1997), and it only attacks small and medium-small fire ant workers. This makes *P. curvatus* an excellent complement for *P. tricuspis,* which only attacks medium and medium-large fire ants (Morrison *et al.*, 1999 a,b). (3) Native fire ants were never as abundant as the imported species currently are (Porter *et al.*, 1988; Porter, 1992; Vinson, 1994), so there is little or no likelihood that native fire ant would simply replace imported fire ants as community-dominating pests. (4) Finally, the Las Flores biotype was originally collected from black fire ants (*S. richteri*) and then reared for several years on red fire ants (*S. invicta*) in quarantine. Even after this time, the Las Flores biotype still preferred black fire ants in choice tests (Porter and Briano, 2000). Field releases of this biotype have resulted in establishment several times on black and hybrid imported fire ants in Mississippi and Alabama (Graham *et al.*, 2003; Vogt and Streett, 2003), but failed seven of seven times on red imported fire ants in Florida (Graham *et al.*, 2003). In contrast, the Formosa biotype of *P. curvatus* was originally collected from red fire ants in northern Argentina. Field releases of this biotype have succeeded four of four times on red imported fire ants in Florida and South Carolina (Vazquez and Porter, unpubl. data; Davis, Pereira and Horton, unpubl. data). These results contradict data from no-choice tests with the Las Flores biotype, which predicted that *P. curvatus* would do as well on black as red imported fire ants (Porter and Briano, 2000). Perhaps the choice tests better indicated whether the flies were able to detect potential hosts at the range of several meters or more, a skill probably not tested in our small no-choice test boxes. Whatever the reason, it appears that the Las Flores biotype from black fire ants was too host specific to succeed in the field on red fire ants. If so, then it almost certainly poses little threat of becoming established on our native fire ants as predicted in spite of the fact that it successfully attacked and developed in native fire ants in laboratory tests (Porter, 2000).

Pseudacteon obtusus We now know, based on AFLP analysis (Kronforst, Gilbert and Folgarait, unpubl.) that the smaller form of *P. obtusus* (Porter and Pesquero, 2001) is, in fact, a separate species. So far, this smaller species has shown no inclination to attack anything except *saevissima* complex ants. The risk of releasing the large form of *P. obtusus* from Herradura and Corrientes, Argentina, is intermediate between releasing *P. curvatus* and the two larger species *P. tricuspis* and *P. litoralis. Pseudacteon obtusus* attacked native fire ants, although at greatly reduced rates. However, an extremely high preference for imported fire ants in host choice tests (over 95%; Porter, unpubl. data) suggests that *P. obtusus* may be very specific to imported fire ants under field conditions. *Pseudacteon obtusus* showed no inclination to attack native ants in other genera.

Other Pseudacteon species Data for the remaining *Pseudacteon* species in Table 1 are mixed, but it is likely that further tests will show that all are at least as host specific as *P. curvatus.* The initial results from *P. nudicornis* and the small species near *P. obtusus* indicate that both species will be highly host specific. The five *P. borgmeieri* flies tested indicate low host specificity (Table 1); however, the failure of this species to successfully parasitize two South American fire ants in the *saevissima* complex (Folgarait *et al.*, 2002b) suggests that it is likely to fail to successfully parasitize the native fire ants in the United States. If *P. wasmanni* (Porter, 1998b) or any of the other species can complete development in native fire ants, then it will be necessary to test them against ants in other genera. A complete set of tests have not been run with *P. wasmanni, P. borgmeieri, P. nocens, P. cultellatus, P. nudicornis*, or the new species near *P. obtusus* because we have not yet been able to rear these species in the laboratory. The low test numbers in Table 1 are due to difficulty in to collecting and transporting adult flies to the United States before they die. As discussed previously, this problem could be partially solved by conducting the tests in South American quarantine facilities, but this would likely require considerable effort and justification to make the necessary arrangements.

GENERAL RISK ASSESSMENT SUMMARY FOR FIRE ANT DECAPITATING FLIES

Field introductions of South American fire ant decapitating flies in the United States began after careful analyses of risks and benefits as elaborated in three Environmental Assessments for field release, which the authors separately prepared with and for officials at USDA/APHIS five, seven, and nine years ago. Below are the conclusions of the risk assessments submitted in support of requests for field-release permits.

1. ***Fire ant decapitating flies will not be a risk to plants, crops, or any agricultural products and may provide many benefits.*** Adult flies are not attracted to fruits or vegetables (Porter, 2000). Immature flies do not develop in or on plants. These flies may also substantially benefit agriculture by reducing fire ant damage in citrus, soybeans, potatoes, corn, sorghum, hay, etc. (Lofgren, 1986).

2. ***Fire ant decapitating flies pose no health risk for humans and may provide considerable health benefits.*** Adult flies are not attracted to humans, human wastes, or human food products. Immature flies pose no threat of developing in human tissues because of their specialized life history. However, if these flies prove to be successful biological control agents, they would be of considerable medical benefit to several hundred thousand people who are severely allergic to even a single fire ant sting (~1% of the population; Vinson, 1997) and to tens of thousands of small children every year who are stung repeatedly by hundreds of fire ants when they accidentally step into the mounds.

3. ***Fire ant decapitating flies pose no health risk to livestock or other domesticated animals and may provide health benefits as well.*** As noted above, these flies are not attracted to vertebrates, nor can they develop in vertebrate tissues (see above). Furthermore they are not attracted to, nor can they develop in, animal excrement. These flies, however, may provide health benefits to livestock by reducing the incidence of fire ant stings, especially to newly born animals.

4. ***The introduction of these flies will not be a risk to native wildlife or any native arthropods except perhaps some ants. Furthermore, they may considerably benefit natural biodiversity.*** Highly specialized ovipositors, oviposition behavior, host preferences, and pupation habits preclude conceivable risks to any organisms except ants. If these flies are able to help reduce fire ant populations, they would considerably benefit natural biodiversity (Porter and Savignano, 1990) and probably the survival of a number of rare or endangered vertebrates.

5. *Pseudacteon species do not present a realistic risk to non-Solenopsis fire ants.* None of the flies tested to date were attracted to other genera of ants in the field, and the few attacks that occurred in the laboratory did not produce any parasitized workers. It is theoretically possible for *Pseudacteon* phorids to switch to ant hosts in different genera because several species have done just that during the process of evolution (Disney, 1994). However, this is only likely to occur in evolutionary time scales of hundreds of thousands of years. Even then, such switches would be limited to a small subset of ants of similar size (Porter, 1998a). There is no conceivable possibility that a fire ant decapitating fly from South America would ever become a generalist parasite of ants.

6. *Several of the Pseudacteon species proposed for release present a real but acceptable risk to Solenopsis geminata, Solenopsis xyloni and other native fire ants.* The primary risk suggested by our specificity testing is that occasional attacks on non-target native fire ant species might occur. Several *Pseudacteon* species can also develop in *S. geminata* and *S. xyloni* under laboratory conditions (Table 1). However, all of these species are much more successful attacking imported fire ants than either of the native fire ant species tested. Furthermore, they also have a strong preference for imported fire ants over native fire ants when allowed to choose. These data justify a conclusion that *Pseudacteon* flies present a much greater risk to imported fire ants than to either of the native fire ants tested. This being the case, the likelihood is that these flies will actually benefit these native fire ant species a rather than harm them because imported fire ants are the primary enemy of native fire ants. Furthermore, risks to native fire ants need to be balanced against the possible benefits of this fly to hundreds of native arthropods and dozens of native vertebrates that appear to be threatened by high densities of imported fire ants. In short, a slight risk from secondary attacks pales in contrast to the benefit of finding an economic, self-sustaining, and target-specific biological control of imported fire ants.

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CHAPTER 14. DETERMINING THE HOST RANGE OF *APHANTORHAPHOPSIS SAMARENSIS*, A SPECIALIZED TACHINID INTRODUCED AGAINST THE GYPSY MOTH

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BACKGROUND OF SYSTEM

DESCRIPTION OF PEST INVASION AND PROBLEM

One of the consequences of the Civil War was the collapse of the cotton industry in the South. Ultimately, this led to idle textile mills in the New England states. A Franco-American scientist, Étienne Léopold Trouvelot, sought to capitalize on this situation by using giant silkworm moths native to North America to develop a sericulture industry (Liebhold *et al.*, 1989). Because a disease caused by a protozoan (*Nosema bombycis* Naegeli) had a devastating impact on the silk industry in Europe (Leggett, 1949), Trouvelot sought to negate this problem by crossing the European gypsy moth, *Lymantria dispar* (Linnaeus), with American silkworm moths, hoping to develop a pathogen-resistant silkworm (Howard, 1930). During the course of his experiments, conducted at his home at 27 Myrtle Street, Medford, MA, some immature stages escaped (Forbush and Fernald, 1896), and the moth began its colonization of North America in 1868 or 1869 (Liebhold *et al.*, 1989). Since that time, literally millions of dollars have been expended in attempts to eradicate, retard the spread, or suppress this invasive pest.

The gypsy moth is probably the most destructive forest and shade tree pest in the northeastern United States, defoliating a record 13 million acres in 1989. It attacks primarily hardwood trees, especially oak, although after the larvae are half-grown they will attack conifers. They usually do not infest ash, black walnut, catalpa, or yellow-poplar (tulip tree). The range of this introduced pest is primarily the northeastern United States, from Maine south to North Carolina and west to Wisconsin. Small, isolated infestations have been reported from California, Tennessee, and Iowa. Male moths have been trapped in a number of other states.

The eggs hatch in late April or early May, with the larvae completing feeding in late June or early July. After feeding, the larvae pupate within loose silken cradles and emerge as adult moths in about two weeks. Shortly after the female emerges, she mates and begins laying eggs on trees, rocks, or other nearby objects. The female, too heavy with eggs to fly, deposits buff-colored clusters of 100 to 1,000 eggs. The current year's egg masses can be found from late July or August until April or May of the following year. The gypsy moth has one generation per year.

When populations reach outbreak levels, gypsy moth defoliation produces adverse ecological effects and economic impacts in both forests and urban-suburban settings (McManus and McIntyre, 1981). Because it defoliates numerous species of shade trees and becomes a severe nuisance pest in urban environments, gypsy moth can be characterized as a "people pest" of the first order. This factor has afforded gypsy moth a high political profile and has driven many of the decisions made in efforts to eradicate or suppress the pest, including efforts at its biological control, which began shortly after the start of the 20th century, when biological control as a discipline was in its infancy (Clausen, 1978). As in the case of most pest problems, there was considerable pressure to obtain a quick solution, and the gypsy moth was no exception; consequently, the overall strategy was to introduce many species of control agents in the hope that one or more of them would suppress the pest. Before 1980, the host specificity of natural enemies introduced against pest insects was not a major consideration. In fact, the polyphagous nature of *Compsilura concinnata* Meigen, a tachinid fly established between 1907 and 1909 (Howard and Fiske, 1911) and now believed to have adverse effects on native giant silkworm moths (Boettner *et al.*, 2000), was considered desirable by early workers because this fly would attack the imported cabbageworm, *Pieris rapae* (L.); browntail moth, *Euproctis chrysorrhea* (L.); and satin moth, *Leucoma salicis* (L.) (Howard and Fiske, 1911; Burgess and Crossman, 1929). A detailed description of earlier (pre-1990) work on classical biological control of the gypsy moth is beyond the scope of this chapter, and the interested reader is referred to the reviews provided by Hoy (1976), Reardon (1981), and Van Driesche *et al.* (1996).

A total of 16 introduced natural enemies became established as a result of these efforts: three predators – *Calosoma sycophanta* (L.), *Carabus auratus* L., and *Carabus nemoralis* Müller (Coleoptera: Carabidae) (Clausen, 1978); 11 parasitoids – *Ooencyrtus kuvanae* (Howard) (Hymenoptera: Encyrtidae), *Anastatus disparis* Ruschka (Hymenoptera: Eupelmidae), *Cotesia melanoscelus* (Ratzeburg) (Hymenoptera: Braconidae), *Phobocampe unicincta* (Gravenhorst) and *Pimpla (=Coccygomimus) disparis* (Viereck) (Hymenoptera: Ichneumonidae), *Brachymeria intermedia* (Nees) (Hymenoptera: Chalcididae), *Monodontomerus aereus* Walker (Hymenoptera: Torymidae), *Compsilura concinnata* (Meigen), *Exorista larvarum* (L.), *Parasetigena silvestris* (Robineau-Desvoidy), and *Blepharipa pratensis* (Meigen) (Diptera: Tachinidae) (Howard and Fiske, 1911; Burgess and Crossman, 1929; Hoy, 1976; Schaefer *et al.*,1989); and two pathogens – gypsy moth nuclear polyhedrosis virus (*Ld*NPV) (Glaser and Chapman, 1913) and *Entomophaga maimaiga* Humber, Shimazu and Soper (Zygomycetes: Entomophthorales) (Andreadis and Weseloh, 1990). The latter species has produced dramatic epizootics in gypsy moth populations in the years after its initial recovery in 1989 (Hajek *et al.*, 1995, 2000; Webb *et al.*, 1999). In addition, *E. maimaiga* appears to have had adverse effects on the guild of larval parasitoids, particularly the univoltine tachinid flies *P. silvestris* and *B. pratensis,* both of which attack late instars of the gypsy moth (Blumenthal and Wilt, 1998). There is some evidence that *E. maimaiga* might not be as effective in the Great Lakes region as in other parts of the gypsy moth distribution (McCullough *et al.*, 2001).

DESCRIPTION OF AGENT PROPOSED FOR INTRODUCTION

Biology of the parasitoid Two independent evaluations of biological control work on gypsy moth were made during the 1990s (Delfosse *et al.*, 1994; Van Driesche *et al.*, 1996). Both recommended that further overseas exploration for natural enemies be focused in non-outbreak or low density populations of gypsy moth. This need prompted us to re-examine *Aphantorhaphopsis* (= *Ceranthia*) *samarensis* as a potential candidate for importation, study, and possible release. This fly, originally described from Russia in 1921 (Sabrosky and Reardon, 1976), was first recovered from gypsy moth in Austria (Fuester *et al.*, 1983). Because only a few puparia were recovered from *L. dispar* during our two-year study, we concluded that it was an occasional parasitoid of gypsy moth. However, a 10-year study conducted by Mills and Nealis (1992) suggested that this species had substantial potential for biological control of gypsy moth. In brief, they experimentally exposed gypsy moth larvae in areas where local gypsy moth populations were at low densities, recollected the hosts, and returned them to the laboratory to rear out the parasitoids. They concluded that *A. samarensis* represented a promising candidate for biological control of gypsy moth in Canada for the following reasons: (1) This parasitoid is able to persist in areas where gypsy moth populations are low and thus presumably has good host searching ability. (2) It responds quickly to local increases in gypsy moth density. (3) It was by far the main parasitoid attacking sentinel larvae exposed in the field, with very high rates of parasitism. (4) Based on photoperiod and temperature conditions in central Europe (same latitude as southern Ontario), if established in the United States, most of the parasitoid's population would be univoltine and not require alternate hosts. (5) Puparia in diapause could be shipped to Canada and overwintered in quarantine. (6) Post-storage emergence rates could be determined under a variety of environmental conditions. Releases were made in Canada (Nealis and Quednau, 1996)

Quednau (1993) worked out the biology of *A. samarensis,* which is briefly summarized as follows. This species hibernates as a pharate adult in the puparium. Newly emerged females mate with older (5-6 day old) males. There is a 10-12 day pre-oviposition period. The egg is deposited directly on the host; hatching occurs immediately, and the neonate maggot rapidly bores into the host (ovolarviposition). The average number of progeny produced by a female over its lifetime is 55. Females live an average of 41 days and deposit their eggs on second and third instars of *L. dispar.* The parasite develops internally, forming a respiratory funnel that produces a characteristic circular scar on the host cuticle. Development in the host takes 6-14 days, and the full grown maggot generally emerges from third or fourth instars, or less frequently, fifth instars. Because *A. samarensis* attacks earlier larval stages than the other univoltine tachinids associated with gypsy moth, interspecific competition with *E. maimaiga* might be less intense.

Source of agent This Palearctic species has a wide distribution in northern and central Europe: Austria (Fuester *et al.*, 1983), France (Mills and Nealis, 1992), Germany (Maier, 1990), Hungary (Mihalyi, 1986), Poland (Sukovata, 2000), and Sweden, as well as the Leningrad and Kubyshev regions of Russia (Herting, 1984). All of the material used in our laboratory tests with North American Lepidoptera came from eastern France (Haute-Saône, Bas-Rhine, and Côte d'Or provinces) and had been reared from *L. dispar*.

Host range in native range of agent Prior to our host range studies, *A. samarensis* only had been recorded from two hosts in Europe, both lymantriids: *L. dispar* (Fuester *et al.*, 1983; Maier, 1990; Mills and Nealis, 1992; Kenis and López-Vaamonde, 1998) and *Orgyia recens* (Hübner) (Mihalyi, 1986). However, this parasitoid has not been reported from other destructive European lymantriids that have been studied extensively: nun moth, *Lymantria monacha* Linnaeus (Komarek, 1937; Fahringer, 1941; Thompson, 1944-1950; Thompson and Simmonds, 1964-1965; Herting, 1976; Mills and Schoenberg, 1985); browntail moth (Burgess and Crossman, 1929; Sisojeviæ*et al.*, 1976); satin moth (Pawlowicz, 1936; Pisica *et al.*, 1978; Drea and Fuester, 1979); rusty tussock moth, *Orgyia antiqua* (L.) (Wellenstein and Fabritius, 1973; Drea and Fuester, 1979; Mills and Schoenberg, 1985); and pale tussock moth, *Elkneria pudibunda* (L.) (Herting, 1960; Wellenstein, 1978).

THE RECEIVING LOCATION

DESCRIPTION OF FAUNA IN AREA OF PROPOSED AGENT INTRODUCTION

Area of proposed release Prior to the completion of our studies (Fuester *et al.*, 2001), *A. samarensis* already had been released in Canada (Mills and Nealis, 1992; Nealis *et al.*, 2002), but permanent establishment of the parasitoid there had not been documented (nor has it been to date). We wished to make releases of this species against gypsy moth in the northeastern United States, but the possibility that *C. concinnata* may have contributed to the reported decline of several saturniid moths in New England (Boettner *et al.*, 2000) and recent recoveries of *P. disparis*, a parasitoid of gypsy moth from the Far East introduced during the 1970s, from non-target species (Schaefer *et al.*, 1989) prompted us to look at the host range of *A. samarensis* more closely before taking action.

Species closely related to target pest There are only seven genera of Lymantriidae in North America, all of which fall in the subfamily Lymantriinae (Ferguson, 1978). Three of the genera (*Lymantria, Leucoma,* and *Euproctis*) fall in the tribe Lymantriini but are monotypic, represented solely by introduced species from Europe (gypsy, brown-tail, and satin moths), all of which can be considered legitimate target pests. The remaining genera fall in the tribe Orgyiini. Two of these are alpine or boreal: *Acsala,* known only from Alaska and the Yukon, and *Gynaephora,* widely distributed in the Arctic but occurring only above the tree line in the Rocky Mountains and northern Appalachians (New Hampshire, Maine, and Quebec). The likelihood of a temperate, sylvan species such as *A. samarensis* attacking species in these genera seems very remote indeed. This leaves two genera of interest – *Dasychira* (16 spp.) and *Orgyia* (10 spp.). At least 11 spp. of *Dasychira* occur in the northeastern United States – *tephra* Hübner, *dorsipennata* (Barnes and McDunnough), *vagans* (Barnes and McDunnough), *basiflava* (Packard), *meridionalis* (Barnes and McDunnough), *cinnamomea* (Grote and Robinson), *leucophaea* (J. E. Smith), *obliquata* (Grote and Robinson), *plagiata* (Walker), *pinicola* (Dyar), and *manteo* (Strecker). About half are minor forest and shade tree pests (Baker, 1972): *basiflava* (dark tussock moth) on a variety of hardwoods; *cinnamomea* on elm; *vagans, meridionalis,* and *tephra* on oaks; and *pinicola* and *plagiata* (pine tussock moth) on conifers. None are considered rare, and all are broadly distributed (Ferguson, 1978). Most of the North American *Orgyia* are western species, and only four occur in the northeastern United States – *antiqua* (L.) (rusty tussock moth), *detrita* Guérin-Méneville, *definita* Packard (definite-marked tussock moth), and *leucostigma* (J. E. Smith) (white-marked tussock moth). All of these except *O. detrita* are considered to be pests of forest and shade trees (Baker, 1972; Drooz, 1985; Johnson and Lyon, 1988; Wallner, 1989). Though not considered a pest and rare in collections, *O. detrita* is widely distributed (Ferguson, 1978), and at least one outbreak has been reported from coastal North Carolina (Drooz *et al.*, 1986).

Species of value as biological control agents Lepidoptera attacking aquatic weeds were not considered to be at risk of attack by *A. samarensis.* A number of Lepidoptera attack common reed, *Phragmites australis* Cavanilles, but all bore within shoots, roots, or rhizomes (Blossey *et al.*, 2002), and it is unlikely that they would be exposed to attack by *A. samarensis.* Two key natural enemies of spotted knapweed (*Centaurea maculosa* L.), the gelechiid *Metzneria paucipunctella* (Zeller) and the cochylid *Agapeta zoegana* L., feed in flower heads and roots (Story, 2002), respectively, and are probably thus protected from attack by *A. samarensis.* The sphingid *Hyles euphorbiae* L., a defoliator of leafy spurge (*Euphorbia esula* L.) (Nowierski and Pemberton, 2002) and cypress spurge (*Euphorbia cyparisseas* L.) (Faubert and Casagrande, 2002), occurs in New York state and is not sequestered within the plant, so it might be subject to attack by *A. samarensis.*

Species of marked conservation value Two endangered butterflies, the Karner blue (*Lycaeides melissa samuelis* Nabokov) and Mitchell's satyr (*Neonympha mitchelli mitchelli* French), occur in the northeastern United States. The first species inhabits meadows and prairies, and the second species, sphagnum bogs. Whereas the known hosts of *A. samarensis* are forest insects, it is expected that this fly will be limited to forest habitats. Because neither of the endangered butterflies occurs in forests, it is anticipated that they will be ecologically separated from *A. samarensis.* While no Saturniidae appear on the United States Fish and Wildlife Threatened and Endangered species list, Boettner *et al.* (2000) have provided evidence that *C. concinnata* can destroy large numbers of several species in the field, and a number of saturniids appear on state lists. For example, buck moth, *Hemileuca maia* (Drury); imperial moth, *Eacles imperialis pini* Mitchener; and Columbia silk moth, *Hyalophora columbia* (S. I. Smith) are listed among the endangered, threatened, and special concern Lepidoptera of Michigan. The monarch butterfly, *Danaus plexippus* L., while rather common in the eastern United States, has precarious overwintering sites in Mexico and has been the subject of recent studies concerning possible hazards presented by genetically modified corn pollen (containing toxins derived from *Bacillus thuringiensis* Berliner) landing on its food plant, milkweed. Therefore, it surely can be considered an icon species.

THE TESTING PLAN: ANALYSIS OF METHODS

Search for Other Hosts in Europe

Our study differed from most other host range studies in that it was not confined to challenging the candidate parasitoid with non-target species in the laboratory; but, in addition, an effort was made to field collect other hosts that might be parasitized by *A. samarensis* in its region of origin. We felt that this would be useful because of the possibility that *A. samarensis* could have been overlooked by previous investigators. Therefore, we made extensive collections of gypsy moth and other Lepidoptera at localities in Europe where *A. samarensis* was abundant (see Fuester *et al.* [2001] for a detailed account of the methodologies employed and results obtained).

TEST LIST FOR HOST RANGE TESTING

The test list of U.S. Lepidoptera selected for host range testing of *A. samarensis* in quarantine appears in Table 1. It was compiled by one of us (RWF) with input from Dale Schweitzer (The Nature Conservancy). The table includes the reasons why the various species were selected. In brief, the lymantriids were selected because they are closely related to the gypsy moth. The Noctuoidea exclusive of Lymantriidae (Noctuidae, Notodontidae, and Arctiidae) were considered somewhat related to gypsy moth. The rest were forest species that belonged to a sensitive group (Saturniidae) or were considered icon species (monarch butterfly). Unfortunately, only about half of the desired species were actually tested, generally because no mated females of *A. samarensis* were available when we had the caterpillars in hand. This was the case for *Amphipyra pyramidoides* Guenee, *Chaetoglaea sericea* (Morrison), *Sericaglaea signata* (French), *Malacosoma disstria* (Hübner), *Datana ministra* (Drury), *Biston betularia cognatoria* (L.), and *Hemileuca maia* Drury. For four other species, we were unable to obtain material to rear – *Ceratomia hageni* Grote, *Pachysphinx modesta* Harris, *Prochoerodes transversata* (Drury), and *Clostera inclusa* (Hübner).

In addition, laboratory tests on host suitability of European Lepidoptera were carried out by inoculating caterpillars with young neonate maggots of *A. samarensis.* No special list of hosts was developed in advance, but inoculations were made on an *ad hoc* basis as host larvae became available.

DESCRIPTION AND RATIONALE OF TESTS RUN

Choice tests We relied mostly on choice tests for our laboratory studies with North American Lepidoptera. We used a choice test format because not all mated females of *A. samarensis* laid eggs when exposed to larvae even though all flies had been held long enough to become gravid. We feared that false negatives could occur if female flies that were not gravid (or were not behaviorally ready to lay eggs) were the ones chosen to be exposed to a non-target species. Quednau and Lamontagne (1998), found that the gestation period of mated females of *C. samarensis* ranges from 7-8 days at 22°C to 17 days at 10-15 °C (12:12 L:D) and, because of variation in the time of day when mating occurs and the metabolism of individual females, not all females in a cohort begin ovolarviposition on the same day. We conducted these tests in a rearing room of the quarantine facility at the United States Department of Agriculture, Agricultural Research Service's Beneficial Insect Introduction Research Unit at Newark, Delaware, at 25°C, 50-60% RH, and a photoperiod of 14:10 (L:D). Screened cages (46 x 33 x 40 cm) with sliding plexiglas doors were used as test arenas. Flies were provided with sponges soaked in distilled water for moisture, and sugar cubes and jelly (Quednau and Lamontagne, 1998) for food. During tests, we exposed 15 gypsy moth larvae (second or early third instars) on a bouquet of red oak and 15 larvae of a nontarget species of similar size on a bouquet of a preferred host plant to two females of *A. samarensis.* Tests lasted 48 h and cages were gently

Table 1. U.S. species proposed for host range tests with *Aphantorhaphopsis samarensis*

Family/Scientific Name	**Common Name**	**Reason Chosen**
Danaidae		
* *Danaus plexippus* L.	Monarch Butterfly	Icon species
Sphingidae		
Ceratomia hageni Grote	Hagen's Sphinx	Forest species
Pachysphinx modesta Harris	Modest Sphinx	Forest species
Saturniidae		
* *Eacles imperialis* (Drury)	Imperial Moth	Sensitive group
* *Actias luna* (L.)	Luna Moth	Sensitive group
* *Automeris io* (Fabricius)	Io Moth	Sensitive group
* *Citheronia regalis* (Fabricius)	Regal Moth	Sensitive group
Hemileuca maja (Drury)	Buck Moth	Sensitive group
Noctuidae		
Amphipyra pyramidoides Guenee	Copper Underwing	Somewhat related
Chaetaglaea sericea Morrison	Silky Sallow	Somewhat related
* *Heliothis virescens* (Fabricius)	Tobacco Budworm	Readily available
Sericaglaea signata (French)	Variable Sallow	Somewhat related
* *Spodoptera exigua* (Hübner)	Beet Armyworm	Readily available
* *Trichoplusia ni* (Hübner)	Cabbage Looper	Readily available
Lymantriidae		
* *Orgyia leucostigma* (J. E. Smith)	White-marked Tussock	Closely related
* *Dasychira vagans* (Barnes and McDonnough)	Variable Tussock	Closely related
Dasychira basiflava (Packard)	Yellow-Based Tussock	Closely related
Lasiocampidae		
Malacosoma disstria (Hübner)	Forest Tent Caterpillar	Forest species
Geometridae		
Biston betularia cognataria (L.)	Pepper & Salt Moth	Forest species
Prochoerodes transversata (Drury)	Large Maple Spanworm	Forest species
Notodontidae		
Datana ministra (Drury)	Yellow-necked Caterpillar	Somewhat related
Clostera inclusa (Hübner)	Angle-lined Prominent	Somewhat related
Arctiidae		
* *Pyrrharctia isabella* (J. E. Smith)	Isabella Tiger Moth	Somewhat related
* *Spilosoma virginica* (Fabricius)	Yellow Wooly Bear Moth	Somewhat related
* *Hyphantria cunea* (Drury)	Fall Webworm	Somewhat related
* *Grammia virgo* (L.)	Virgin Tiger Moth	Somewhat related
* *Estigmene acrea* (Drury)	Salt Marsh Caterpillar	Somewhat related

*Species actually tested

atomized with distilled water at least twice a day. When test periods were completed, larvae were reared to determine if parasitism had occurred. Caterpillars were reared in ventilated plastic cages (12 [h] x 12 [dia] cm) with false bottoms similar to those described by Loan and Holdaway (1961) so that any maggots of *C. samarensis* that emerged would drop to the bottom and not be injured by any unparasitized caterpillars. After test exposures, larvae of gypsy moth were fed with an artificial diet while non-target species larvae were fed small bouquets of their usual host plant (or artificial diet if the species came from a laboratory culture). Hosts were reared to the pupal stage or until death occurred and categorized as parasitized, unparasitized (healthy), diseased, desiccated, or dying of unknown causes. Hosts dying before reaching the pupal stage were dissected to see if parasitization had occurred.

No-choice tests A limited number of no-choice tests were run by Philip Kingsley in the quarantine facility at the United States Department of Agriculture, Animal and Plant Health Inspection Service's Methods Development Center at Otis Air National Guard Base in Massachusetts. In each case, only five test larvae per species per trial were offered to females of *A. samarensis.* The oviposition cages (arenas) were similar to those described by Quednau (1993).

Host Suitability Tests Tests on host suitability were performed on several European species of macrolepidoptera by artificially inoculating larvae of *L. dispar* and non-target species with mature eggs of *C. samarensis* that had been dissected from uteri of gravid females three weeks or more in age and then placed on potential hosts with a watercolor brush. Females of butterflies and moths were netted or caught by light trapping and caged to obtain eggs. Many females were caught and some of them laid eggs, but most of the eggs did not hatch. Thus, very few caterpillars were available for testing. Inoculations were performed by restraining a host larva with pins, removing the hairs from the 9th and 10th body segments, and placing a freshly eclosed maggot on the host integument with a moistened brush. Maggots were kept damp with Ringer's solution while they searched for an entry site. Once a site was chosen, entry through the integument took 30 seconds. All larvae were reared on a natural host plant until *A. samarensis* emerged or pupation occurred. One month after oviposition, live larvae that had not pupated were dissected.

TEST RESULTS AND INTERPRETATIONS

Results of Field Collections in Europe

In addition to some 20,360 larvae of *L. dispar*, over 850 larvae in at least 54 other species in 11 families were collected and reared over a five-year period from field sites in Europe. Out of 103 larvae in five species of other Lymantriidae, only two, one of *L. monacha* and one of *O. antiqua*, yielded puparia that could not be distinguished from those of *A. samarensis*, but no adults emerged, so new host records could not be claimed with certainty. No *A. samarensis* was obtained from any of the remaining centrifugal groupings, which included the Noctuoidea other than Lymantriidae (492 specimens in 22 species), Heterocera other than Noctuoidea (135 specimens in 26 species), or Rhopalocera (121 specimens in seven species). Even if one assumes that the puparia recovered from *L. monacha* and *O. antiqua* were *A. samarensis,* overall parasitization rates across all years and sites for gypsy moth, other lymantriids, and Lepidoptera other than lymantriids would be 8, 2, and 0%, respectively. Thus, gypsy moth was obviously the chief, if not the only, host utilized by *A. samarensis* at our field sites. We feel that this is an important finding because the results reflected what was actually going on in the field in habitats favorable to *A. samarensis.*

RESULTS OF LABORATORY TESTING IN NORTH AMERICA

Assessment of overall testing success A summary of the trials (=replicates) pairing *L. dispar* with North American species of Lepidoptera appears in Table 2. Choice and no-choice tests were conducted with 14 and two native species, respectively.

In the choice tests, 1-5 trials were run for each species, depending upon the numbers of caterpillars and parasitoids available. Unfortunately, females of *A. samarensis* failed to attack any hosts (including the *L. dispar* control) whatsoever in nearly 40% of all trials, rendering the results inconclusive. We did not anticipate this high rate of failure of *A. samarensis* to attack *L.*

Table 2. Numbers of successful and unsuccessful laboratory trials attempted for native species of North American Lepidoptera exposed with *L. dispar* to two females of *A. samarensis*, 1997-1998.

Native species tested	No. of trials attempted	No. of trials unsuccessful[a]	No. of trials successful[b]
	Choice tests		
D. plexippus	2	1	1
A. luna	2	0	2
E. imperialis	1	1	0
C. regalis	1	1	0
P. isabellaa	1	0	1
S. virginica	5	3	2
H. cunea	5	0	5
G. virgo	4	0	4
E. acrea	2	2	0
Dasychira sp. prob. *vagans*	1	0	1
O. leucostigma	4	0	4
S. exigua	1	0	1
H. virescens	5	3	2
T. ni	4	4	0
Totals	38	15	23
	No-choice tests		
A. io	3	0	3?
A. luna	1	0	1

[a] Neither the test (native) or control (*Lymantria dispar*) species were parasitized
[b] Either the test, control species, or both were pararasitized

dispar because we were using two female flies instead of just one, as per Quednau and Lamontagne (1998) in their rearing protocol. In 10 of the 14 species tested, one or more trials were successful in that at least some control hosts (*L. dispar*) were attacked, and it was possible to draw inferences as to whether the non-target larvae were likely to be acceptable hosts for *A. samarensis.* In those cases where no hosts were parasitized, we concluded that either the females were not gravid or not behaviorally ready to lay eggs. The four species in which all trials were inconclusive were the saturniids *Citheronia regalis* (Fabricius) and *Eacles imperialis* (Drury), the arctiid *Estigmene acrea* (Drury), and the noctuid *Spodoptera exigua* (Hübner).

In the no-choice tests, none of the non-target species were parasitized in any of the trials, but at least some of the *L. dispar* in each trial (as evidenced by the production of puparia) had been attacked by the females of *A. samarensis.* However, even in these cases, the results cannot be considered conclusive because of the possibility that the female flies used in nontarget species cages might not have been gravid or might not have been behaviorally ready to eggs

Assessment of host range The results of those trials that we considered successful are presented in Table 3. Successful choice and no-choice tests were run with ten and two native species, respectively. In one case, *Actias luna* (L.), both choice and no-choice trials were run. In every choice test between gypsy moth and non-target species except one, *A. samarensis* attacked the gypsy moth but not the non-target species (Table 3). We concluded that all of these species were outside of the host range of *A. samarensis.* Females of *A. samarensis* attacked only one non-target species, the white-marked tussock moth, *Orgyia leucostigma* (J. E. Smith), another lymantriid. In this case, substantial numbers of hosts were attacked, yielding about two puparia per parasitized host, so we concluded that *O. leucostigma* lies within the host range of *A. samarensis.* The only other lymantriid tested, *Dasychira* sp., probably *vegans* (Barnes and McDunnough), was not parasitized.

Concerning the no-choice tests, three paired trials were run with *Automeris io* (Fabricius) and *L. dispar.* The results were similar in all trials: no larvae of *A. io* yielded puparia of *A. samarensis,* but 10 puparia were obtained from gypsy moth (Table 3). At least one female fly in each trial and arena with gypsy moth was gravid, attacking the target pest. Although it is conceivable that all females exposed to *A. io* were not gravid, it seems unlikely. Therefore, we suspect that *A. io* does not lie within the host range of *A. samarensis.* In the remaining test, one trial was run with test larvae of *A. luna* and *L. dispar,* each species in a different arena. No larvae of *A. luna* yielded puparia of *A. samarensis,* but three puparia were obtained from gypsy moth. Although it is possible that the *A. samarensis* exposed to *A. luna* were not gravid, the results are at least consistent with the results in the choice test with the same species (Table 3), so we believe that *A. luna* does not lie within the host range of *A. samarensis.*

RESULTS OF HOST SUITABILITY TESTS IN EUROPE

Successful development of fly larvae implanted in field-collected caterpillars occurred only in gypsy moth (Table 4). Dead parasitoid larvae were found in three arctiids, one nemeobid, and one noctuid. No maggots successfully penetrated *H. euphorbiae,* a sphingid, which was the only biological control agent tested.

Table 3. Results of successful tests involving exposures of *L. dispar* with selected native species of North American Lepidoptera to two gravid females of *A. samarensis*, USDA-ARS, Newark, Delaware, 1997-1999

Native species tested	Host plant of native species tested[a]	Hosts attacked (puparia recovered)		Native species within or outside host range
		Native species	***L. dispar***	
		Choice tests		
D. plexippus	*Asclepias syriaca* L.	0	5(6)	outside
A. luna	*Juglans nigra* L.	0	9(10)	outside
P. isabella[b]	Mixed Graminaceae	0	4(14)	outside
S. virginica	*Betula populifolia* Marshall	0	2(2)	outside
H. cunea	*Prunus serotina* Ehrhart	0	40(73)	outside
G. virgo	*Lactuca sativa* L.	0	30(72)	outside
Dasychira vagans?	*Quercus prinus* L.	0	4(4)	outside
O. leucostigma	*Acer rubrum* L.	20(45)	5(5)	within
S. exigua	*Pyrus malus* L.	0	1(1)	outside
H. virescens	*Rosa multiflora* Thunberg	0	5(6)	outside
Totals		**20(45)**	**105(193)**	
		No-choice tests[c]		
A. io	*Quercus* sp.	0	?(12)	outside?
A. luna	*Quercus* sp.	0	?(3)	outside

[a] All exposures of control species, *L. dispar*, made on *Quercus rubra* Linnaeus.
[b] Only four test larvae per species instead of 15.
[c] In no-choice tests, only five test larvae per species instead of 15.

SUMMARY EVALUATION

Overall Synthesis

The results of all four approaches used—review of the literature, field collections of Lepidoptera in a favorable habitat for the candidate natural enemy within its native range, laboratory host range tests on North American species, and artificial inoculations to assess host suitability—led us to the conclusion that the host range of *A. samarensis* is restricted to the family Lymantriidae,

Table 4. Lepidopterous larvae inoculated with young *A. samarensis* maggots and results of rearings or dissections.

Species and Family	**No. larvae inoculated**	**No. of *A. samarensis* reared**	**No. of dead maggots found by dissection**
Agriades glandon (Prun.) - Lycaenidae	3	0	0
Hamearis lucina (L.) - Nemeobidae	2	0	0
Macrothyacia rubi (L.) - Lasiocampidae	1	0	0
Hyles euphorbiae (L.) - Sphingidae	2	0	0
Peridea anceps (L.) - Notodontidae	1	0	0
Callimorpha dominula (L.) - Arctiidae	17	0	3
Eilema deplane (Esper) - Arctiidae	4	0	4
Lithosia quadra (L.) - Arctiidae	3	0	1
Lymantria dispar (L.) - Lymantriidae	38	20	—[a]
Mamestra brassicae (L.) - Noctuidae	2	0	7

[a] Non-parasitized *Lymantria dispar* were not dissected.

probably only to the genera *Lymantria* and *Orgyia*. Because the only *Lymantria* species in North America is the pest *L. dispar*, and all four species of *Orgyia* in the eastern United States are native pests (Baker, 1972; Drooz, 1985; Drooz *et al.*, 1986; Johnson and Lyon, 1988; Wallner, 1989), the host range of *A. samarensis* seemed specific enough to justify release, and an Environmental Assessment was submitted by USDA-APHIS to the State of Pennsylvania. This resulted in a finding of no significant impact, and releases of *A. samarensis* were made by personnel from the Pennsylvania Department of Environmental Resources, Bureau of Forestry. The technical results of our studies were published in a peer-reviewed journal (Fuester *et al.*, 2001).

COMPLETENESS OF ASSESSMENT

It would have been desirable to do more tests on North American Lepidoptera, especially in the genus *Dasychira* and possibly of other Noctuoidea. We only did a few no-choice tests with *A. samarensis*, but to have relied on such tests exclusively could have given rise to false negative tests because of the parasitoid's refractory behavior. Sequential choice tests, with flies presented first to nontarget species and then shortly thereafter to the target pest could have been used to provide an appropriate control, but were not. Because we used long exposure times (48 hours), we thought our choice tests would provide the parasitoids ample opportunity to attack the non-target species offered. In the case of *O. leucostigma*, the only other acceptable North American host besides gypsy moth, we saw a female of *A. samarensis* attempt oviposition within a minute of introduction to the test arena. Extended direct observation of fly behavior in choice tests might have shown whether attention paid to the higher ranked host was preempting discovery and assessment of the nontarget host.

As of December 2003, *A. samarensis* has not been recovered from gypsy moth at release sites in Canada or the United States, so field studies have not been run to detect its presence in non-target species. If *A. samarensis* is recovered, such studies will be implemented.

RECOMMENDATIONS FOR FUTURE WORKERS

Because entomophagous insects can behave abnormally in the laboratory, attacking hosts that are not normally attacked in nature (Simmonds, 1944), we agree with Greathead (1995) that field studies in the country of origin to determine an agent's natural host range are useful in assessing the risk that a candidate species for introduction might present to non-target organisms in the new environment. One of the problems in such an approach, of course, is the reality of community structure. Figure 1 shows the frequency distribution of the caterpillars of macrolepidoptera we recovered at our study sites in Europe. There are a large number of species (in fact, most) that are represented by only a few specimens – too few to allow for quantitative estimates of incidence of parasitism. Nevertheless, we feel that the information acquired was useful for three reasons. First, it was realistic: all hosts were collected in the field, where they had been naturally exposed to foraging females of the parasitoid. Second, all of the hosts collected were indigenous, suggesting that the host range of *A. samarensis* was stable and had not expanded to include invasive species. Third, it demonstrated that *A. samarensis* was not widely polyphagous: otherwise, we should have made numerous recoveries scattered over the various taxa collected. Our approach might be rendered more useful by making exposures of other hosts to augment sample sizes for host species of special interest, especially those related to the target pest or favoring the same host plants. In any case, we feel that this ap-

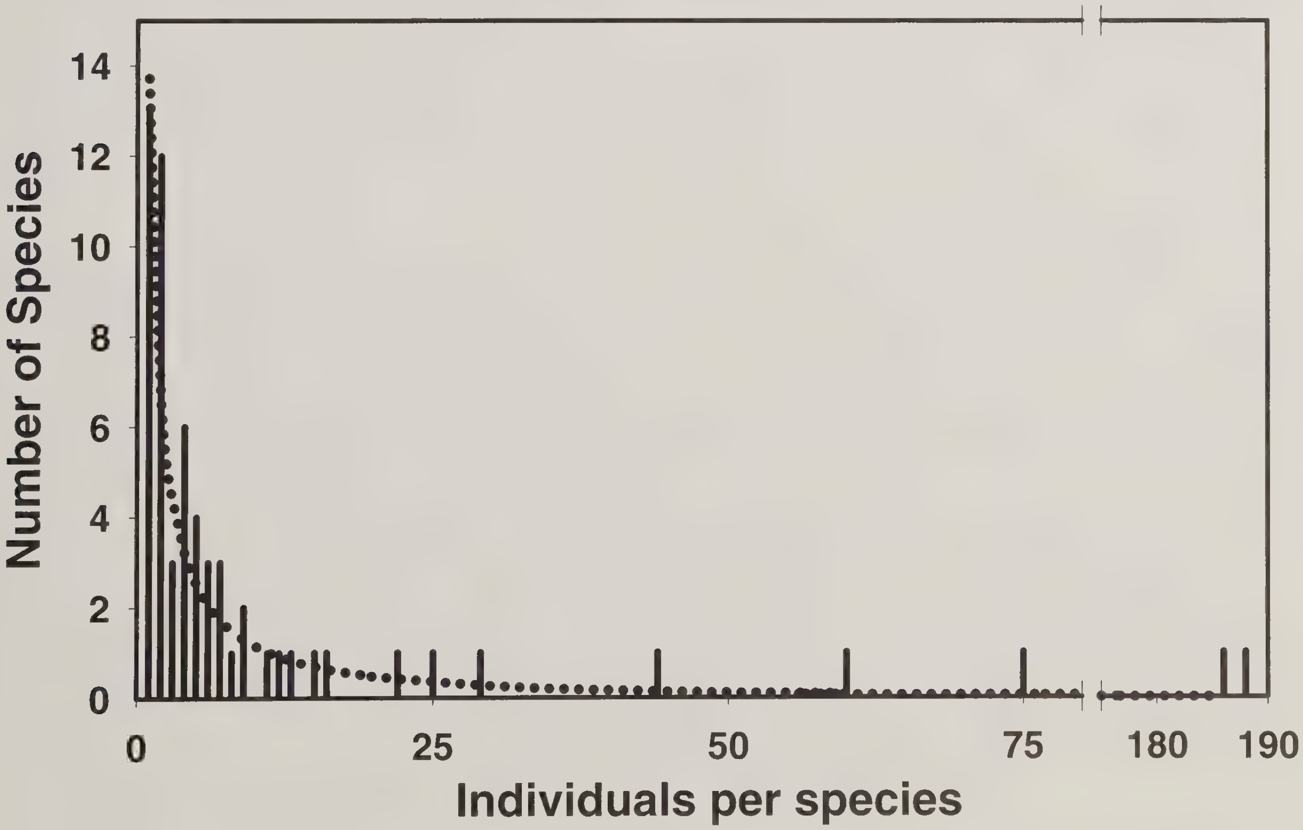

Figure 1. Frequency distribution of species of Macrolepidoptera, with different numbers of individuals, recovered at *A. samarensis* study sites in France and Switzerland, 1993-1999.

proach merits greater emphasis and that agencies involved in foreign exploration for natural enemies could commit more resources to it.

Another problem was the difficulty of obtaining and rearing potential non-target hosts for the laboratory screening tests. Most of the species selected for testing were not of economic interest, so laboratory colonies did not exist. We were successful in capturing and rearing some, but not all, of the species we sought. In addition, many forest Lepidoptera only have one generation a year, and we frequently had caterpillars available when the parasitoids were not or vice versa. A more flexible approach to developing a list of non-target species for testing might be to designate genera instead of species, at least in those cases where there is no specific concern for a particular species. The enlistment of amateur entomologists to aid in the search for test species might also prove useful.

Many of the females of *A. samarensis*, even though incubated long enough after mating to be gravid, didn't lay eggs. Consequently, many of our test caterpillars were wasted, which was a significant problem with the non-target species, which were usually in short supply. The failure of flies to lay eggs might have been mitigated by using more flies per trial, increasing the likelihood that at least one would attack the control host.

Another way to solve this problem might have been to use sequential no-choice tests instead of choice tests. This involves alternately offering a female of parasitoid, first, a given nontarget test species, then the target pest, then the same nontarget test species again. Dissection of the first series of target pest caterpillars would provide data to determine whether a particular parasitoid was able to oviposit in a known host (the pest). This approach has the advantage (over regular choice tests) that the target pest (presumably a preferred species) is not present with the nontarget test species and thus cannot divert the parasitoid from attacking it, should it prove to be a less desirable but acceptable host. Making the first exposures to the nontarget species (rather than the target pest) avoids conditioning the parasitoid to a preferred host. Similarly, this design has an advantage over no choice tests, because the ability of each individual parasitoid to oviposit is determined during the test.

We probably could have made greater use of the artificial inoculation approach in assessing the risk to non-target species, but it would have involved much more rearing of the latter. It might have been used profitably to get more information on the suitability of Saturniidae, a group of special conservation interest; several species are available commercially because they are showy and popular with collectors. This approach is not practical for most endoparasitoids because inoculation requires the use of hypodermic needles or some other procedure that would be traumatic to the host. With this particular system, it seemed to work well because the neonate parasite larva could enter the host on its own.

Our biggest problem in this research was the difficulty in rearing and handling the parasitoid. The rearing, performed by the late Dr. Kingsley, is very labor intensive. However, difficulty in rearing or otherwise handling a natural enemy, while important in mass rearing, should not be a prime consideration in classical biological control.

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CHAPTER 15. PREDICTING THE FIELD PREY RANGE OF AN INTRODUCED PREDATOR, *RODOLIA CARDINALIS* MULSANT, IN THE GALÁPAGOS

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BACKGROUND

This chapter describes and discusses the procedures used to evaluate the potential threats of a predator, *Rodolia cardinalis* Mulsant, to the conservation of the insect fauna of the Galápagos Islands, a UNESCO world heritage site and biosphere reserve. Due to their late discovery and settlement by humans, the Galápagos Islands are the least altered of any oceanic archipelago (Tye *et al.*, 2002). However, Galápagos species are increasingly at risk because of increased human migration to the islands and the associated rise in alien species introductions (Snell *et al.*, 2002 a,b). To date, more than 450 species of introduced insects have been recorded as established in the archipelago (Causton *et al.*, unpub.). The liberation of *R. cardinalis* in 2002 to mitigate damage to native plants from the invasive scale *Icerya purchasi* Maskell marked the first recorded intentional introduction of an insect into the Galápagos. The risk of introducing a species that might turn out to be a hindrance rather than a help to ecosystem conservation provoked much debate among scientists in the Galápagos. The costs and benefits of introducing *R. cardinalis* were analyzed carefully following the presentation of a risk assessment (Causton, 2001, 2003) that included the feeding range studies that are discussed here.

TARGET PEST: *ICERYA PURCHASI*—A THREAT TO ENDANGERED FLORA

Icerya purchasi (Homoptera: Margarodidae) is a cosmopolitan and polyphagous pest that feeds on at least 200 plant species from many families. The damage to its hosts includes stunting, branch deformation, premature abscission of fruits and leaves, dieback, and even death of the entire plant. Commonly known as the cottony cushion scale, *I. purchasi* is native to Australia but has invaded over 80 countries, primarily through movement of plants or fruit. It is best adapted to tropical and semi-tropical regions (Hale, 1970).

Since it was introduced to the Galápagos in 1982 (on incoming ornamental plants), *I. purchasi* has colonized 15 islands in the archipelago. The spread of this scale insect has been attributed to human activity and dispersal by wind currents (Roque-Albelo and Causton, 1999). Damage by this sap feeding insect was first noticed in 1996, a particularly dry year. Since then, 62 native or endemic species have been recorded as host plants of *I. purchasi*. Sixteen of these species are listed as threatened in the IUCN (International Union for the Conservation of Nature) Red List of Threatened Species, of which six are classified as Endangered or Critically Endangered (Causton, 2001, 2003). Furthermore, the scale's debilitating effect on some plant species, especially those that are already threatened, appears to indirectly affect endemic Lepidoptera that rely exclusively on these species as food sources (Roque-Albelo, 2003).

In 1996, the Charles Darwin Foundation (CDF) and the Galápagos National Park Service (GNPS) identified *I. purchasi* as an invasive species whose impacts required immediate mitigation. Chemical control was not a possible option because of the wide distribution of this pest and because of the impacts pesticides would have on native invertebrates. At the request of the GNPS, the CDF formed a technical advisory committee to evaluate the possibility of employing biological control for the first time on the Galápagos Islands (Causton *et al.*, 2004). The committee concluded that studies should be carried out by entomologists at the Charles Darwin Research Station (CDRS), the operative arm of the CDF, to determine (1) whether the detrimental impact of *I. purchasi* on the native flora and fauna was sufficient to merit the introduction of a biological control agent and (2) what risks to the Galápagos biota might result from introducing a natural enemy of *I. purchasi*. The coccinellid beetle *R. cardinalis* was selected as the most suitable biological control agent because of its success in controlling *I. purchasi* in many parts of the world.

RODOLIA CARDINALIS: THE SOLUTION—BUT IS IT SAFE?

Rodolia cardinalis, otherwise known as the vedalia beetle, is believed to be native to Australia (Prasad, 1989). After the successful use of this beetle to control *I. purchasi* on citrus in California in the 1880s, *R. cardinalis* was introduced into over 60 countries. It has successfully established on various continents and islands (Bennett *et al.*, 1985; Caltagirone and Doutt, 1989). Because most releases of *R. cardinalis* took place before host testing protocols had been developed, and because of a general absence of post-introduction monitoring, relatively little was known about its feeding range before we initiated our studies.

Many authors have suggested that the range of prey attacked by *R. cardinalis* is narrow and limited to Margarodidae (fluted scales and ground pearls), yet on reviewing the literature and the labels on museum specimens, we found that there was only limited evidence of stenophagy (Causton *et al.*, 2004). This was principally because few autoecological studies had been carried out on this biological control agent. Although some laboratory studies had tested the response of *R. cardinalis* to a few alternate prey such as aphids and mealybugs (Balachowsky, 1932; Kuwana, 1922), these trials did not reveal much about *R. cardinalis*' feeding range. This was because only some of the stages of the predator were tested and crucial information was not included in the description of the methods such as the number of individuals tested and whether they had prior feeding experience, what kind of test arena was used, and whether no-choice or choice tests were used.

Most records of development or feeding by *R. cardinalis* are limited to prey in several genera of Margarodidae, suggesting specialization on this family of scale insects. However, we also found some unconfirmed prey records of *R. cardinalis* feeding on other families of Homoptera, including a dactylopid in its native range of Australia (Frogatt, 1902) and aphids, mealybugs, and armored scales in other parts of the world (R. Booth, pers. comm., 1998; Muma, 1953-54, 1955 as cited by Hodek, 1996; Thompson and Simmonds, 1965). Even though evidence was not available to substantiate these records, we had to assume that *R. cardinalis* might present a risk to these groups. Intraguild predation occurring between *R. cardinalis* and other scale insect predators was also a possibility.

As a result of this preliminary research, the Galápagos advisory committee concluded that there were insufficient data available to fully demonstrate that *R. cardinalis* would not threaten any Galápagos species. At the request of the committee, entomologists at CDRS carried out an assessment of the risks associated with the introduction of *R. cardinalis* that included feeding range tests with potential non-target species.

TESTING LOCATION

Tests were carried out at the CDRS in the Galápagos Islands following a cost-benefit analysis of the economical and logistical advantages of conducting tests "in situ" compared with contracting an organization outside the Galápagos Islands to do the work. Costs were reduced considerably by carrying out the tests in the Galápagos even though it meant building an insect containment facility for this purpose. Not only was it cheaper, but we were also able to test a wider range of species by avoiding the need to ship non-target Galápagos species to another testing location. Tests were carried out from 1999-2000.

DEVELOPMENT OF A LIST OF TEST SPECIES

STEP 1: SELECTION OF CRITERIA FOR IDENTIFYING POTENTIAL NON-TARGET SPECIES

To establish the list of non-target species that needed to be tested, we first had to set criteria to define which Galápagos species were most likely to be harmed by the introduction of *R. cardinalis* (Figure 1). To do this, literature on the ecology of *R. cardinalis* and other coccinellid species was reviewed, in particular literature pertaining to foraging behavior, habitat and feeding range. This information provided a preliminary estimate of which families might be used as prey, what characteristics of a prey species might stimulate foraging, and which other species might be directly and indirectly affected. Another important source of information was literature that referred to methods for conducting feeding range tests on predators and parasitoids (e.g., Sands, 1998; Kuhlmann *et al.*, 1998; Barratt *et al.*, 1999; Keller, 1999; Kirk and Thistlewood, 1999; Sands and Van Driesche, 2000; Lopez and Kairo, 2003). However, because only a handful of entomophagous species have been tested, we also reviewed the literature available for testing weed biological control agents (e.g., Wapshere, 1974; Harley and Forno, 1992).

The following criteria were chosen for selecting species for inclusion in the feeding range tests (see Table 1):

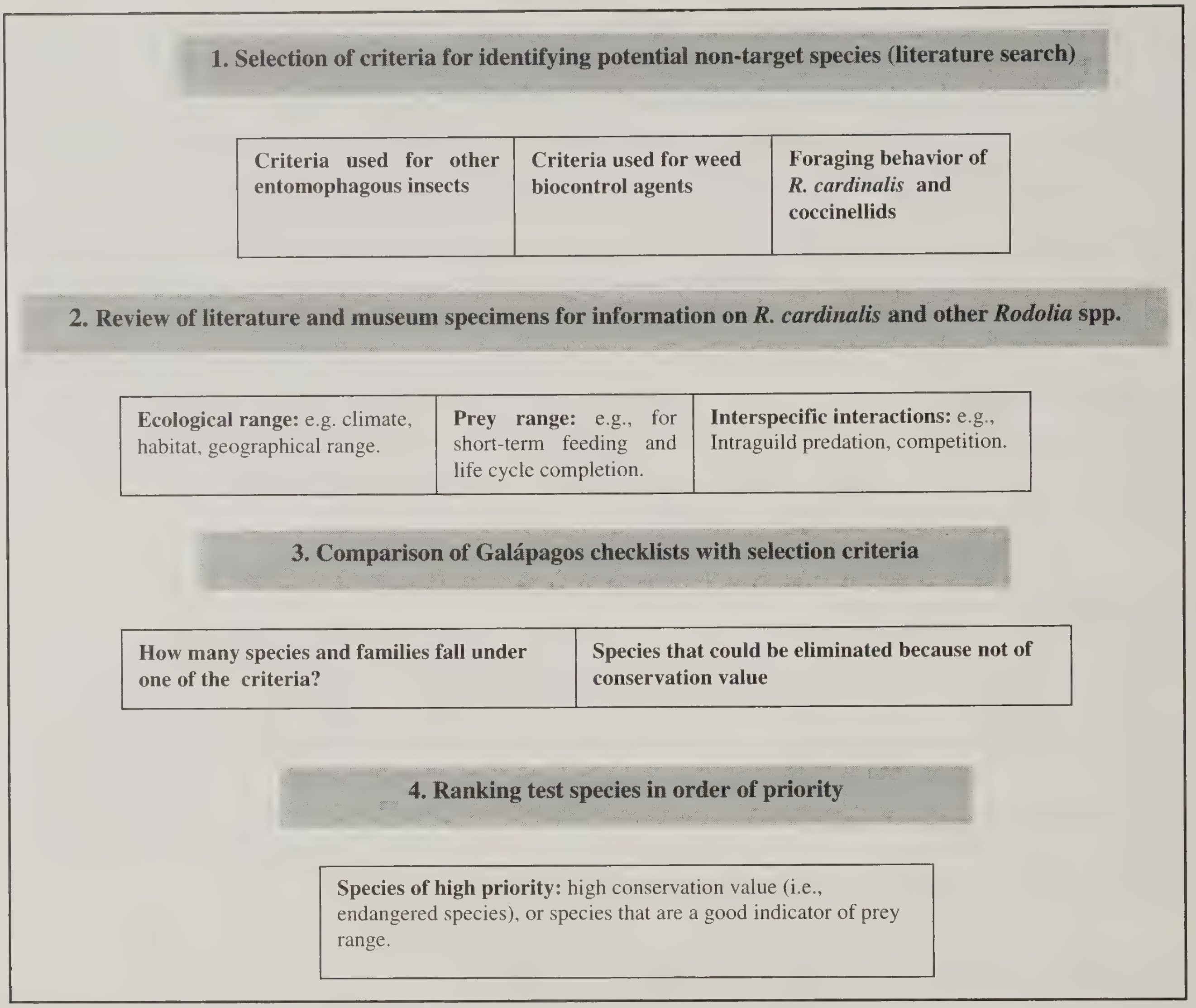
1. Selection of criteria for identifying potential non-target species (literature search)
Criteria used for other entomophagous insects
Criteria used for weed biocontrol agents
Foraging behavior of *R. cardinalis* and coccinellids
2. Review of literature and museum specimens for information on *R. cardinalis* and other *Rodolia* spp.
Ecological range: e.g. climate, habitat, geographical range.
Prey range: e.g., for short-term feeding and life cycle completion.
Interspecific interactions: e.g., Intraguild predation, competition.
3. Comparison of Galápagos checklists with selection criteria
How many species and families fall under one of the criteria?
Species that could be eliminated because not of conservation value
4. Ranking test species in order of priority
Species of high priority: high conservation value (i.e., endangered species), or species that are a good indicator of prey range.

Figure 1. Summary of important considerations for developing a list of test species.

1. **Species closely related to *I. purchasi* or the Margarodidae** Centrifugal testing (Wapshere, 1974), widely used for weed biological control agents, assumes that the closer the species is taxonomically to the target pest, the more likely it is to be attacked.
2. **Species previously reported as prey for any *Rodolia* species** Because coccinellids that prey on scales are known to exhibit restricted feeding ranges (Dixon, 2000), the feeding habits of congenerics were also considered to be a useful indicator of the potential feeding range of *R. cardinalis.*
3. **Species morphologically or physiologically similar to *I. purchasi*** Olfactory and visual cues such as wax filaments produced by scale insects are often necessary to prompt coccinellid foraging and oviposition, (Merlin *et al.*, 1996; Dixon, 2000). We assumed that such prey characteristics would influence prey selection by *R. cardinalis.*
4. **Species that live in close proximity to prey of *R. cardinalis*** Species of insects, in particular Homoptera or endangered insects, were considered to be at risk if they occupied niches close to *I. purchasi.* Furthermore, natural enemies that fed either on the pest *I. purchasi* or

Table 1. Groups of Galápagos species potentially affected by *Rodolia cardinalis*.

Selection criteria (relative to target pest)	**Nature of impact**	**Potential prey based on the literature**	**Groups (number of species present in Galápagos)**
Same family	Predation	Margarodidae	*Margarodes similis*
Closely related families	Predation	All Coccoidea	Ortheziidae (1), Eriococcidae (2), Pseudococcidae (7), Diaspididae (3)
Other Homoptera reported as *Rodolia* prey	Predation	Aphididae, Aleyrodidae	Aphididae (3)
Species morphologically similar to *I. purchasi*	Predation	Scale insects with waxy covering	Ortheziidae, Eriococcidae, Pseudococcidae
Unrelated species in close proximity to *R. cardinalis* prey	Competition and predation	Neuroptera, Diptera (Cecidomyiidae, Syrphidae), Hymenoptera, Coccinellidae	Chrysopidae (1), Coccinellidae (10)
Species of conservation value	Toxic reactions produced by feeding	Insectivorous vertebrates	Finches (13), mocking birds (4), warbler (1), lizards (1)

other taxa identified as potential prey of *R. cardinalis* were also considered to be at risk due to competition or intraguild predation. A higher probability of encounter was likely if natural enemies were very common.

5. **Invertebrates of conservation value that might interact with *R. cardinalis***

STEP 2: REVIEW OF LITERATURE AND MUSEUM SPECIMENS FOR *RODOLIA* SPECIES

Sources of information Field studies of *R. cardinalis* in its native range and in countries where it has been introduced, although valuable, were not financially possible. Our knowledge of its feeding range came from the literature and information supplied by museum curators, coccinellid specialists, and biological control practitioners. Databases and search engines on the Internet were also reviewed. Particularly useful sources were Scalenet, CAB Abstracts, and Biological Abstracts. Unfortunately, many of the museum records that we found were not substantiated by published information to confirm whether *Rodolia* species actively fed on the prey listed or were able to complete development on it. We questioned the accuracy of some literature prey records because Hodek (1996) in his review of coccinellids found that adult behavior has often been misinterpreted. He pointed out that finding an adult coccinellid on top of a scale insect is not necessarily an indication that it is feeding on this species. The honeydew of scale insects is often used for short-term survival by coccidophagous insects when their prey is not available. Some host records may reflect insects found feeding on honeydew or merely resting on a branch that happened to have a scale infestation. We decided, however, that the fact that a species or taxa had been reported as prey meant that it should be considered as a potential non-target species.

Information was also sought on the ecological and geographical range of *R. cardinalis* to determine the likelihood of overlap with potential non-target species in the Galápagos. In addition to this, climate in the Galápagos was compared with that in the beetle's native range using the program Climex (Skarratt *et al.*, 1995). Contrary to our predictions, we were unable to find any climatic matches. At the time, we only had 10 years of rainfall data from one Galápagos island available to us (which included an El Niño event) and because the precipitation data used were highly variable, our data were probably not representative of climate in the Galápagos.

Prey records More than half of the 73 existing prey records that we found for *Rodolia* species were simply observations taken from museum labels or other unsubstantiated notes. Feeding range studies have only been carried out for three *Rodolia* species (*Rodolia fumida* Mulsant, *Rodolia iceryae* Janson, and, *Rodolia limbata* Blackburn) that have been used as biological control agents. These tests found that larval development was only possible on margarodids, and in one case only on *Icerya* species (Rasheed *et al.,* 1986; Kairo and Murphy, 1995; Brancatini, unpub.). Except for one unconfirmed record of feeding on mites, our review indicated that *Rodolia* species are restricted to feeding on Homoptera, with 13 out of 21 *Rodolia* species feeding only on margarodids. The remaining species fed on margarodids but were also recorded as preying on other scale insects from the superfamily Coccoidea (in families such as coccids, dactyliiopids, diaspidids, ortheziids, and pseudococcids), in addition to whiteflies and aphids.

For *R. cardinalis* specifically, we found 20 prey records, and this information indicated that the vedalia beetle's prey range was almost entirely restricted to the Coccoidea (Margarodidae, Pseudococcidae, Diaspididae and Dactyliiopidae), with the exception of two unconfirmed reports of feeding on aphids. We found that 12 of the prey records were for margarodids in the genera *Auloicerya, Crypticerya, Drosicha, Gueriniella, Icerya, Monophlebus, Monophlebulus,* and *Palaeococcus* (Koebel, 1893 cited in Balachowsky, 1932; Kuwana, 1922; Balachowsky, 1932; Anon, 1939 cited in Kairo and Murphy, 1995; Moutia and Mamot, 1946; Bartlett, 1978; Gery, 1991; Ragab, 1995; Mendel *et al.*, 1998; V. Brancatini, pers. comm., 2002, 2003). Prey records for *R. cardinalis* also included two genera of mealybugs (Pseudococcidae) – *Maconellicoccus* and *Rastrococcus*; two genera of armored scales (Diaspididae) – *Aspidiotus* and *Selanaspidus*; one dactyliopiid – *Dactylopius*; and one aphid – *Aphis* (Frogatt, 1902; Muma, 1953-54, 1955 as cited by Hodek, 1996; Thompson and Simmonds, 1965; R. Booth, pers. comm., 1998). Prey recorded in *R. cardinalis'* native range were in the genera *Icerya, Monophlebus, Monophlebulus,* and *Dactylopius. Rodolia cardinalis* has been reported to complete its lifecycle on three genera of Margarodidae (several *Icerya* species, *Palaeococcus* and *Gueriniella*), although it appears that in genera other than *Icerya* life cycle completion is only possible if egg masses are eaten (Balachowsky, 1932; Mendel and Blumberg, 1991). Adults can survive for long periods (up to three months) eating pollen and nectar in the laboratory (V. Brancatini, pers. comm., 1999).

Ecological range *Rodolia cardinalis* is adapted to a wide range of climatic regimes (Bodenheimer, 1951). Biological control with this agent has succeeded in countries with temperate, tropical, or desert climates, suggesting that it would adapt to most parts of the Galápagos if food were available.

Interspecific interactions In the laboratory, larvae of *R. cardinalis* have been observed to kill and or displace larvae of *R. iceryae*, even when target prey were available (Mendel and Blumberg, 1991). Predation may have been involved in the displacement by *R. cardinalis* of congeneric species (*Rodolia koebelei* Oliff and *Rodolia amabilis* Gorham) that fed on *I. purchasi* in California and India (Subramanian, 1953; Bartlett, 1978).

In general, the prey range of *R. cardinalis* and other *Rodolia* species appears to be restricted to Homoptera, specifically scale insects, whiteflies, and aphids. Although one record of feeding on mites was found, mites were not placed on the test list because this record seemed highly doubtful given the known feeding range of the genus *Rodolia*. Other species that might be eaten or displaced by *R. cardinalis* were the natural enemies of potential prey. Because of *R. cardinalis*' tolerance to a wide range of habitats, we concluded that species on the test list might be at risk in any above-ground habitat in Galápagos.

STEP 3: COMPARISON OF GALÁPAGOS CHECKLISTS WITH SELECTION CRITERIA

Checklists for Galápagos Homptera, especially Coccoidea, were found to be incomplete with virtually nothing recorded about species distribution, their host plants, or population status. Consequently, field surveys were carried out in 1999 and 2000 to collect needed information. The discovery of at least four species new to science confirmed our suspicions about the deficiencies of the list. New test species were added to the list even after feeding range experiments had started, and it is likely that the list will grow as new areas in the archipelago are surveyed. A database of these species was compiled.

A list was compiled of all Galápagos species that might serve as prey or otherwise be harmed by *R. cardinalis* (see Table 1). Information was sought on the status of each of these species (e.g., endangered, endemic, native or introduced), their distribution, habitats, abundance, host ranges, and their natural enemies. Following this, we used a process of elimination to exclude any species that were introduced (only native and endemic species were considered of conservation value) or were unlikely to come into contact with *R. cardinalis*, such as gall makers and subterranean species. Based on these considerations, several families were dropped from the test list, including soft scales (Coccidae) and whiteflies (Aleyrodidae).

Ultimately, species from five families of Coccoidea (14 species) and the family Aphididae (3 species) were considered potential non-target prey of conservation value (Table 1). Although host records suggest that *R. cardinalis* is specialized to feed on scale insects, we included aphids in the test list because several records of aphids as prey were found in the literature. We also included three species of Coccoidea that were considered unlikely to be prey because (1) they probably live underground (*Margarodes similis* Morrison and *Pseudococcus insularis* Morrison) or (2) were introduced species (*Paracoccus solani* Ezzat and McConnell). Field studies on *M. similis* confirmed that it lives underground, but this species was retained in the test list because of its taxonomic closeness to the target pest.

Very little is known about the prey ranges of natural enemies of Galápagos Homoptera. A literature search determined that coccinellids, syrphids (Diptera), Neuroptera, and some Lepidoptera are predators of scale insects and aphids in other parts of the world. Galápagos checklists were reviewed and compared with these species, and a list of potential non-target species was compiled. This list was supplemented by field surveys. In addition, *I. purchasi* populations were monitored for natural enemies for three years.

Only two generalist insect species were found preying on *I. purchasi*: the possibly endemic neuropteran *Ceraeochrysa cincta* (Schneider) and larvae of the moth *Pyroderces rileyi* Walsingham (Cosmopterigidae). The latter species is a new record for the Galápagos, discovered while we were running the feeding tests. It is thought to be an introduced species (Landry, 2001). We do not know for sure whether it fed on detritus (its preferred dietary preference) or was using *I. purchasi* for food. Laboratory studies confirmed our field observations that none of the ten species of Galápagos coccinellids use *I. purchasi* as prey, although one species – *Cycloneda sanguinea* L. – was observed feeding on the honeydew of *I. purchasi* and might interact with *R. cardinalis*. However, encounter rates between *R. cardinalis* and the other species of coccinellids were thought to be fairly high, as all the species are suspected to be coccidophagous or aphidophagous and could occupy habitats that were close to the target prey of *R. cardinalis*. Very little is known about other natural enemies associated with Galápagos Homoptera. During our field surveys we did not collect any native parasitoids or find any predators associated with native Coccoidea or aphids. However, our field trials were limited. Cecidomyiids were collected from two pseudococcids (*P. solani* and *Pseudococcus* n. sp. #6) during the feeding range tests, but it is not yet known if these flies were predators or scavengers.

Rodolia cardinalis might use nectar and pollen as a temporary, alternative food source when prey are scarce and might therefore interact with native pollinating insects in the Galápagos. However, we did not consider this group to be at risk because most insect pollinators in the Galápagos do not specialize on particular plant groups, and thus would not directly compete with *R. cardinalis* for resources. Furthermore, a high proportion of flowering plants do not require insect pollination (McMullen, 1993).

Based on our analysis of the check lists and the feeding behavior of *R. cardinalis* and other *Rodolia* species, we did not consider it necessary to include any additional invertebrate species of conservation value. However, because some toxicity experiments have demonstrated that at least one species of coccinellid (*Coccinella septempunctata* L.) is toxic to vertebrates (Marples *et al.*, 1989), ornithologists were concerned about the potential effect on insectivorous birds and lizards. Accordingly, some such species were included in the test list. Those experiments are discussed elsewhere (Causton, 2003; Lincango and Causton, unpub.).

STEP 4: RANKING TEST SPECIES IN ORDER OF PRIORITY

Because of limited funding and the high costs associated with collecting from other islands in the archipelago, we considered it necessary to identify which of the potential non-target species were most important to test according to their conservation value or importance as an indicator of the prey range of *R. cardinalis*. Because information on the status and ecology of most of these potentially "at risk" non-target species was non-existent, we used host plant distribution as an indicator of their distribution and abundance. Species of highest priority were the endemic species with a small distribution (i.e., those found on a single island) and specialized feeders with a small host range, especially those that are closely related to *I. purchasi* or feed on rare plant species that are also attacked by *I. purchasi* (Table 1). Species with high scores included pseudococcids, eriococcids, and ortheziids. *Margarodes similis* was also considered a priority because of its close relationship to *I. purchasi.*

DEFINING TESTING PROCEDURES

In order to fully assess the risks of introducing *R. cardinalis*, our studies needed to respond to three questions.

- Could *R. cardinalis* complete development on other insect species in the Galápagos?
- Are any *R. cardinalis* stages able to switch between prey and feed temporarily on native insects and if so, what degree of population impact do they have?
- Could intraguild predation occur between *R. cardinalis* and natural enemies of scale insects?

Guidelines for defining test procedures and the methods used to assess the prey range of *R. cardinalis* are summarized in Figures 2 and 3.

STEP 1: LOCATING A SOURCE OF *R. CARDINALIS*

Adult *R. cardinalis* were donated by CSIRO Entomology in Brisbane, Australia, from a colony that had been screened and found free of pathogens or parasitoids. The colony originated from beetles collected near Brisbane, Queensland. Our colony of *R. cardinalis* was maintained in the quarantine facility at CDRS and was fed on field-collected *I. purchasi* and honey.

STEP 2: BACKGROUND RESEARCH FOR CHOOSING A TEST PROCEDURE

Our goal was to use stages of the predator, test species, and environmental conditions that would most accurately predict the field prey range of *R. cardinalis* in the Galápagos. Achieving this goal required information about the ecology and biology of *R. cardinalis,* as expressed in the following questions:

- Does *R. cardinalis* oviposit on its prey or elsewhere;
- Do confined spaces or any other factors stimulate oviposition in the absence of the host;
- Are olfactory, tactile or any other environmental cues needed to prompt oviposition and foraging, such as specific plant chemicals and morphological features;
- At what age is beetle oviposition highest and how long is the oviposition period;
- Are all larval stages mobile;
- What stages of *R. cardinalis* feed on prey that might be valuable native species;
- Which is the most voracious feeding stage;
- Are any stages cannibalistic?
- What stages of prey does *R. cardinalis* feed on;
- Does *R. cardinalis* feed on parasitized prey; and
- Could prior feeding experience influence prey selection?

1. Locating a source for *R. cardinalis*

Pathogen & parasite free: Need for screening.	**Genetic variability:** Test from different locations.

2. Background research for choosing test procedures (literature search & laboratory studies)

Biology and ecology of *R. cardinalis* and coccinellids: especially foraging behavior and host location.	**Factors that could influence the test outcome:** Cannibalism, random egg laying, target prey chemicals, parasitization.

3. Selection of *R. cardinalis* stages for testing

Stages that are a threat to potential non-targets: All stages that feed.	**Stages that are key for testing:** e.g. show whether life cycle is possible on non-targets and whether can lay eggs.	**Feeding experience of life stage:** Conditioned versus naïve individuals.	**Hunger level :** Avoid testing satiated or stressed individuals, or individuals on the point of pupating or dying.

4. Use of field-collected versus laboratory-reared test species

Logistical and quarantine issues: Facilities for cultivating host plants (inc. those from other islands). Personnel costs.	**Issues of contamination:** Ensure that test species are not parasitized or infected by pathogens.	**Timing:** Test species need to be newly collected at onset of experiment.	**Simulating field conditions:** Test species on potted plants versus cuttings.

5. Choice of prey life stages and host plants for tests

Use stages that are consumed by *R. cardinalis*: All stages.	**Ensure host plant does not influence *R. cardinalis*:** i.e, cuttings, trichomes, plant chemistry.

6. Choosing an appropriate test environment

Type of test arena and size: Not too small to avoid disrupting adult host location, and not too big for neonates that are relatively immobile and need to find the prey before they starve.	**Environmental conditions that are suitable for *R. cardinalis*:** e.g., daylength, temperature, humidity, lighting conditions that do not affect behavior.

7. Definition of test design and protocols

Type of test: e.g., No-choice v. choice.	**Parameters (dependent on prey size):** e.g., Survival, development, feeding and oogenesis.	**Controls:** With target prey and/or no prey.	**No. insects per replicate:** Issues of cannibalism and need for mated beetles.	**No. of trials and replicates:** acceptable level of variability.

8. Preventing contamination with semiochemicals

In the test arena: Residues on containers	**Effects of *I. purchasi* on naïve individuals.**	**Influence of host plant volatiles.**

9. Data analysis

When are test results valid?: dependent on survival of controls fed the target prey.	**Which statistical method to use?**

Figure 2. Summary of important considerations for defining test procedures.

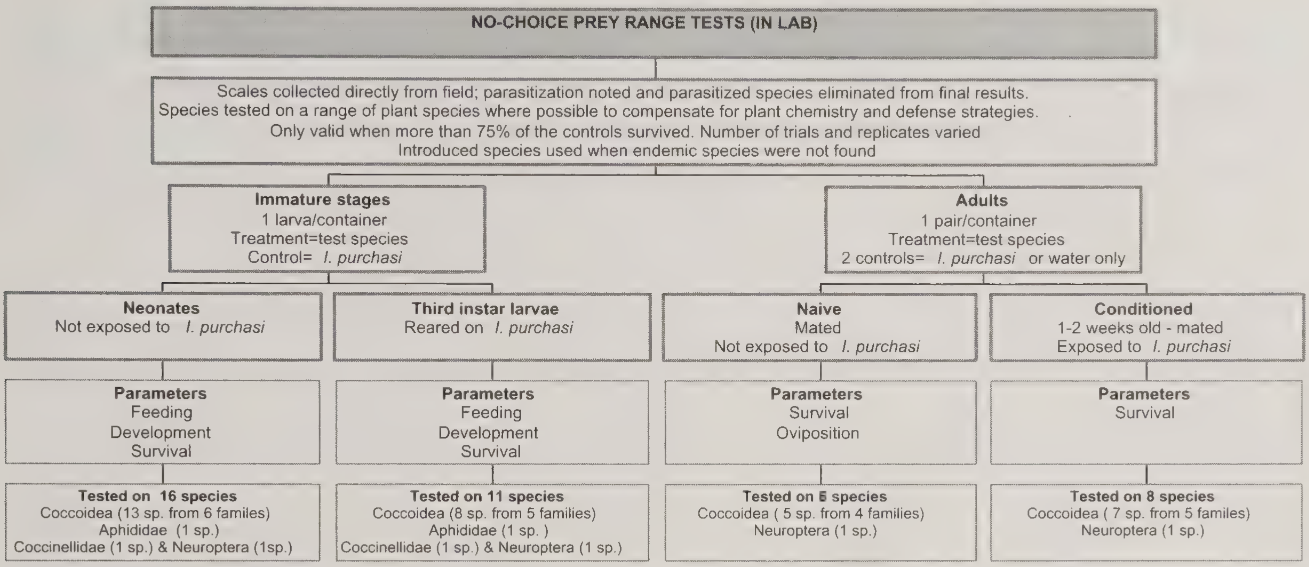
NO-CHOICE PREY RANGE TESTS (IN LAB)
Scales collected directly from field; parasitization noted and parasitized species eliminated from final results.
Species tested on a range of plant species where possible to compensate for plant chemistry and defense strategies.
Only valid when more than 75% of the controls survived. Number of trials and replicates varied
Introduced species used when endemic species were not found
Immature stages
1 larva/container
Treatment=test species
Control= I. purchasi
Adults
1 pair/container
Treatment=test species
2 controls= I. purchasi or water only
Neonates
Not exposed to I. purchasi
Third instar larvae
Reared on I. purchasi
Naive
Mated
Not exposed to I. purchasi
Conditioned
1-2 weeks old - mated
Exposed to I. purchasi
Parameters
Feeding
Development
Survival
Parameters
Feeding
Development
Survival
Parameters
Survival
Oviposition
Parameters
Survival
Tested on 16 species
Coccoidea (13 sp. from 6 familes)
Aphididae (1 sp.)
Coccinellidae (1 sp.) & Neuroptera (1sp.)
Tested on 11 species
Coccoidea (8 sp. from 5 families)
Aphididae (1 sp.)
Coccinellidae (1 sp.) & Neuroptera (1 sp.)
Tested on 6 species
Coccoidea (5 sp. from 4 families)
Neuroptera (1 sp.)
Tested on 8 species
Coccoidea (7 sp. from 5 familes)
Neuroptera (1 sp.)

Figure 3. Summary of procedures used for feeding range trials of *Rodolia cardinalis.*

Step 3: Selection of *R. cardinalis* Stages for Testing

Both adults and larvae of *R. cardinalis* are entomophages and were considered potential threats to non-target species in the Galápagos. Literature and our preliminary studies indicated that *R. cardinalis* lays eggs on or near *I. purchasi* and larvae are initially weak, suggesting that adults define the prey range of recently emerged larvae. Although recently eclosed larvae are only likely to be a threat to a non-target species if the adult has selected it for oviposition, we found that adults that had consumed the target prey (conditioned adults) laid eggs in empty test arenas and that oviposition could not be used as a reliable parameter for testing prey selection. First instar larvae were therefore chosen to determine whether non-target prey could support complete development.

Mature larvae and adults were also selected for testing because our preliminary studies showed that they were voracious feeders and very active, and both of these stages had the potential to encounter other prey species. Prasad (1990) found that adults have a capacity to move over long distances in the field, increasing the probability that they could be found outside the range of its target prey. Although temporary foraging on non-target species is considered acceptable and sometimes necessary for sustaining population numbers of the agent when its target prey is low (e.g., Sands, 1997; Sands and Van Driesche, 2000), in a conservation context such as in the Galápagos, short term feeding by a voracious predator may have considerable impact on non-target species, especially on already threatened endemic species.

Because coccinellids are unable to develop eggs until they have fed on a prey that is nutritionally adequate (Matsuka *et al.*, 1982; Frazer, 1988), we also considered naïve adults in tests of the suitability of non-target species to support oogenesis.

Hunger level and condition of life stage When choosing the stage of a predator for prey range testing, it is also important to ensure that individuals are at a point in their life cycle when they would consume food. For example, it would have done no good to test fourth instar larvae that are on the point of entering the prepupal stage or to test adults that were past peak egg laying, as old adults required less food and died quickly when starved. Consequently, we tested late second and early third instars, which were active and readily consumed prey. Deciding how old adults should be for testing proved to be more complicated. According to Cressman (1930), female beetles eat the most in the first third of their adult lives, following their preoviposition period of 3 to 28 days. However, in preliminary trials, we had observed that the survival rates of adults that had been removed from the target prey varied with age. To ensure that adults were exposed to non-target prey at an age when they would exhibit maximal feeding and to provide a sufficiently long exposure period to the test species, we conducted trials to evaluate the effects of eliminating *I. purchasi* from the diet of *R. cardinalis* adults after 3 days, 1 week, 2 weeks, or 4 weeks after beetle emergence. Ten replicates were tested in each trial, and each replicate consisted of a 9 cm dia. petri dish with a newly emerged female-male pair and two adult female *I. purchasi.* Using an ANOVA followed by a least significant difference LSD means separation process (using the SPSS system in Norusis, 1993), it was determined that beetles removed from a *I. purchasi* diet after 3 days ($P < 0.001$) or one week ($P < 0.05$) lived longer than did beetles that had fed on *I purchasi* for four weeks. Females lived significantly longer ($P < 0.001$, $\overline{X} = 5.7$ days, SD = ± 1.8, n = 38) than males ($\overline{X} = 4.4$ days, SD = ± 2.2, n = 38) when the results were pooled across age classes. Female longevity may have been dependent on reproductive output, with survival in the absence of prey declining in proportion to the number of eggs already laid (see Dixon, 2000). Because we were interested in assessing the prey range of beetles that had sufficient prior feeding experience on the target pest, we decided to test beetles fed on *I. purchasi* for 1 to 2 weeks.

We also asked whether prey selection by the different stages of *R. cardinalis* would be influenced by previous feeding on *I. purchasi,* and if so, might recently emerged larvae and adults that had never been exposed to the target prey behave differently and perhaps eat prey that conditioned adults would reject. To test this hypothesis, naïve, unfed neonate larvae were tested instead of first instar larvae that had already fed on *I. purchasi.* Recently emerged, naïve adults were also tested.

Hunger levels can also influence the outcome of feeding experiments. Satiated individuals often do not respond quickly to prey, while naïve (unfed) individuals may become weak and uninterested in feeding if not tested immediately. In our experiments, conditioned adults were separated from *I. purchasi* and given water but no food for 1-2 days. This was not done when mature larvae were assessed: these were transferred directly to the test arena from containers stocked with *I. purchasi.* Eggs were checked first thing in the morning and regularly throughout the day so that neonates were exposed to a test species soon after emerging. Sluggish individuals were not selected for testing. Naïve adults were kept in plastic containers for a day following their emergence to ensure that they had mated and would be able to lay eggs in the event that they fed on a suitable host.

The rearing conditions of the colony also influenced the state of the life stages used in the trials. An adequate food supply and small number of *R. cardinalis* in each rearing container were important factors in ensuring that beetles were healthy. Crowded containers produced smaller individuals, which, in some coccinellid species (e.g., Booth *et al.*, 1995), reduces fecundity.

In summary, neonates were tested for life cycle completion on a non-target species and to assess conditioning due to prior prey consumption. Mature larvae were used to test their ability to switch between prey species. Naïve adults were used to test their ability to develop and deposit eggs after feeding on non-target species and assess whether or not previous prey contacts influence prey selection. Conditioned adults were used to test adult's ability to switch between prey species. All life stages were tested in separate experiments.

STEP 4: USE OF FIELD-COLLECTED VERSUS LABORATORY-REARED TEST SPECIES

At an early stage, we concluded that the advantages of testing field-collected insects far outweighed testing laboratory-reared individuals. Too little was known about the non-target prey and their host plants and how to cultivate the host plant and use them to rear colonies of test species in the laboratory. In addition to this, because some of these species were found only on islands other than the one we were working on, it would have involved rearing the species under quarantine conditions, which was not logistically or economically possible.

The principal disadvantages of using field-collected insects were that the test species needed to be collected just before the experiments were started and did not survive long once they were collected. This limitation coupled with the need for specific *R. cardinalis* stages made conducting experiments difficult. Another disadvantage of using field-collected prey was that, in the event that results were not significant or were invalid, it was difficult to repeat the experiments until new collections could be made. The post-El Niño conditions prevalent at the time of the trials had lowered the numbers of most of these species, making subsequent collections difficult. Nor could we test adults under simulated field conditions by using potted plants in large cages.

There was also the possibility that some field-collected material would be parasitized or contaminated by pathogens that might not be detected until experiments were underway. However, our surveys showed that few endemic or native species had associated parasitoids or pathogens. Throughout our trials, only three prey species were parasitized (14% of the Homoptera tested), two of which were introduced species while the third was of unknown origin. Although *R. cardinalis* has been known to eat parts of *I. purchasi* parasitized by the dipteran *Chryptochaetum iceryae* Williston in times of prey scarcity (Quezada and Debach, 1973), we decided that it was better to eliminate any test species that were parasitized or diseased. This was in part because little was known about the response of *R. cardinalis* to the presence of other parasitoids. As a precautionary measure, test material and any additional material that wasn't used in the trials were reared after the trial to check for parasitoids. Additionally, two prey species were found to be infected by fungi and were excluded from the final analysis.

STEP 5: CHOICE OF PREY LIFE STAGES AND HOST PLANTS FOR TESTS

Life stages In principle, we wanted to test all life stages of each test species because all stages of *I. purchasi* are consumed by *R. cardinalis.* Early instars of the test species were always included in tests with neonates because neonates' mouthparts may be unable to penetrate the tougher integuments of older stages of some species. In practice, however, the life stages that were tested depended on what was available at the time (see Tables 2 and 3). Test prey were supplemented every three days to ensure that there was a sufficient food supply and plants were fresh.

Table 2. Suitability of potential non-target prey for the development of immature stages of *R. cardinalis.*

Test prey Species [a, b]	**Development of *R. cardinalis* larvae**					
	Neonates[c]			**Third instars[c]**		
	Feeding	**Development**	**n**	**Feeding**	**Development**	**n**
Ortheziidae (Homoptera)						
Orthezia insignis (I)	—	—	15	—	—	10
Orthezia sp. (?)	—	—	21	Nt	Nt	Nt
Margarodidae (Homoptera)						
Margarodes similis (E) (cysts) •	—	—	88	—	—	26
M. similis (emerged females) •	+	—	94	+	—	3
Pseudococcidae (Homoptera)						
Antonina graminis (N?)	—	—	57	—	—	45
Pseudococcus n. sp. # 2 New sp. •	—	—	20	—	—	14
Pseudococcus n. sp. # 3 New sp. •	—	—	44	—	—	22
Pseudococcus sp. (?)	—	—	26	—	—	17
Paracoccus solani (N?) •	—	—	15	Nt	Nt	Nt
Eriococcidae (Homoptera)						
Eriococcus papillosus (E) •	—	—	69	—	—	15
Coccidae (Homoptera)						
Saissetia coffeae? (I)	—	—	11	Nt	Nt	Nt
Parasaissetia nigra (I)	—	—	20	Nt	Nt	Nt
Diaspididae (Homoptera)						
Selenaspidus articulatus (I)	—	—	20	—	—	31
Aspidiotus excisa (I?)	—	—	15	Nt	Nt	Nt
Aphididae (Homoptera)						
Sitobion sp? (E?) • (all stages except eggs)	—	—	69	—	—	25
Coccinellidae (Coleoptera)						
Pentilia sp. (E?) • (mature larvae, pupae and adults)	—	—	8	—	—	28
Chrysopidae (Neuroptera)						
Ceraeochrysa cincta (E?) • (eggs not tested on third instar larvae)	—	—	26	—	—	24

[a] All stages tested unless indicated;
[b] (E) = endemic; (I) = introduced; (N) = native; • = high risk potential prey of conservation value;
[c] — = negative response; + = positive response; Nt = not tested

Food plants Attempts were made to reduce the effects of plant chemistry and plant defenses on the outcome of the tests. Where possible, several food plants were used for test species that used more than one genus as a resource, and plant species that are toxic to insects were avoided (see Step 8). Additionally, we tried to use whole leaves rather than parts of leaves because the chemistry of plants that are cut may be altered and affect prey selection (see Palmer, 1999). We also tried to avoid using species with trichomes and pronounced pubescence that might influence the foraging behavior of the prey, as we had observed that neonates found it hard to walk on some of these species. Furthermore, several authors (e.g., Eisner *et al.*, 1998; Gamarra *et al.*, 1998) have found that coccinellids can be killed or lacerated by trichomes.

Table 3. Survival (number of days) of "conditioned" and "naïve" adult *R. cardinalis* fed on a test prey species compared with individuals given only water (NC).

Test prey species[a]	**Survival (days ± SD)**[b]							
	Naïve				**Conditioned**			
	Test	**n**	**NC**	**n**	**Test**	**n**	**NC**	**n**
Margarodidae (Homoptera)								
Margarodes similis (E) (emerged female)•	10.5 ± 3.8**	10	3.8 ± 1.0	10	5.8 ± 4.3	10	3.1 ± 0.5	10
M. similis (cysts)•	5.5 ± 1.3	10	4.7 ± 1.3	10	2.8 ± 0.3	10	3.4 ± 0.4*	10
	7.8 ± 1.1	11	7.6 ± 2.0	11	Nt		Nt	
Pseudococcidae (Homoptera)								
Paracoccus solani (N?)	6.7± 0.9*	12	5.4 ± 1.0	11	2.0 ± 1.6	17	1.9 ± 0.7	17
	Nt		Nt		3.0 ± 0.7	17	2.9 ± 0.8	17
Pseudococcus sp. #3 New Sp.•	Nt		Nt		3.6 ± 1.2	14	2.8 ± 0.8	13
Pseudococcus sp. #6 New Sp.•	3.9 ± 0.8	8	4.8 ± 1.3	7	2.0 ± 0*	5	1.2 ± 0.4	5
Eriococcidae (Homoptera)								
Eriococcus papillosus (E)•	5.9 ± 1.8	9	4.6 ± 1.4	10	4.2 ± 1.0*	4	2.3 ± 0.6	3
Coccidae (Homoptera)								
Ceroplastes rusci (I)	6.3 ± 1.1	9	6.4 ± 1.7	9	4.1 ± 0.6	7	3.8 ± 0.9	7
	Nt		Nt		4.4 ± 0.5	4	3.9 ± 0.2	4
Diaspididae (Homoptera)								
Aspidiotus excisa (I?)	Nt		Nt		3.1 ± 0.7	13	3.4 ± 0.6	13
Chrysopidae (Neuroptera)								
Ceraeochrysa cincta (E?)•	2.5 ± 1.5	16	NA		3.6 ± 1.3	5	NA	
	Nt		Nt		1.2 ± 0.4	6	NA	

[a] (E) = endemic; (N) = native; (I) = introduced; • = potential prey of conservation value

[b] Sample means compared using independent samples t-test for data with equal variance and Mann-Whitney U test in the event of unequal variation. NA = Not applicable, * = significant ($P<0.05$), ** = highly significant ($P<0.001$)

STEP 6: CHOOSING AN APPROPRIATE TEST ENVIRONMENT

Type of test arena and size Because neonates are virtually immobile, we used a small test arena to guarantee that the predator would encounter the non-target prey. Eppendorf tubes were found to be too big (mouth = 1 cm dia., 4.2 cm high), but were acceptable when the area was reduced by inserting a plug made from Kimwipes® and leaving a 1 cm long space for the larval movement (Figure 4). This methodology was based on similar experiments with *R. limbata* (V. Brancatini, pers. comm., 1999). One of the problems with using this method was that larvae would sometimes burrow into the plug. Orienting the tubes narrow end down reduced this problem. We did not put any water in the containers because preliminary trials showed that even the smallest drop drowned larvae.

Late instar larvae and adults were tested in 9 cm dia. petri dishes (Figure 5). Studies on other entomophagous coccinellids suggests that proximity to the prey stimulates foraging (Samways and Wilson, 1988; Dixon, 2000), and we concluded that the use of a small arena should not disrupt prey location cues. Previous studies with *R. cardinalis* indicated that it would mate and lay eggs in containers of this size (Matsuka and Watanabe, 1980; Ragab, 1995).

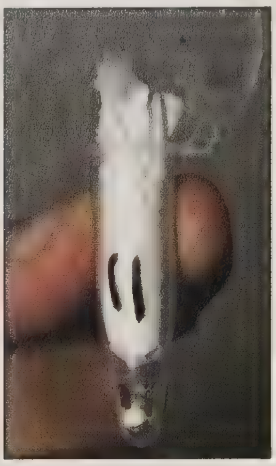

Figure 4. Eppendorph tubes were used to test neonate larvae. This photo shows a positive control using *I. purchasi* and a *R. cardinalis* larva. Photo: Heidi Snell. (UGA1295010)

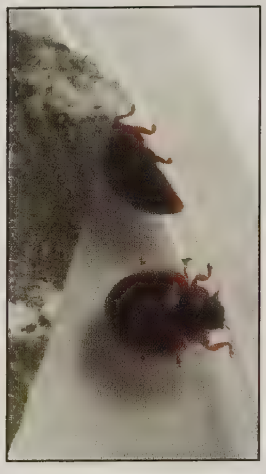

Figure 5. Eggs of an endemic mealybug tested against *R. cardinalis* adults. Indeterminate numbers were used because of their small size. Photo: Heidi Snell. (UGA1295009)

Environmental conditions All trials were conducted at 24-26 °C, 60% average relative humidity, and 12:12 L:D photoperiod. We found that these were acceptable conditions for *R. cardinalis.* Fluorescent bulbs with high frequency electronic ballasts (1500 hz) were used to avoid promoting irregular insect behavior (A. Cross, pers. comm., 1999).

Step 7: Definition of Test Design and Protocols

Type of test – no-choice versus choice We selected no-choice tests because we were primarily interested in seeing if *R. cardinalis* would feed and survive on non-target species rather than in demonstrating differences in predator preference among prey species. Responses in tests of starved larvae or adults to a non-target species (the treatment - T) were compared with the response of individuals offered the target prey (the positive control). Tests thus created an "eat it or die" situation. Although, there was some risk of false positives (feeding on a species that *R. cardinalis* would not normally feed on under field conditions), we felt that there were fewer external factors in this design that might affect prey selection. In choice tests, the presence of semiochemicals from the target prey or another prey can lead the predator to ignore an alternative test prey, inducing a false negative result. Furthermore, use of no-choice tests allowed us to quickly eliminate those species not eliciting feeding from the list of potential prey. This allowed us to screen a larger number of prey species.

Parameters and frequency of measurements To score responses in our no-choice tests, we measured predator survival (number of days alive) to determine if naïve or conditioned adults could feed on non-target prey. For predator larvae, we measured both survival and development (the presence of larval molts). Although molts might suggest feeding, larvae chosen for tests could be close to molting when they were placed in the test arena, and caution should be used in interpreting such events. If feeding was seen, it was recorded, but the number of prey eaten could not be measured because prey were small and numerous, and were continuously emerging from pupae and eggs during the experiments. The number of fecal pellets deposited by adult predators was initially counted but was not used in the analysis because both starved naïve and starved conditioned beetles produced a small number of feces in some trials. In addition, we recorded the number of eggs deposited by adults.

Notes on the behavior of *R. cardinalis* (e.g., location of beetle in the test arena, degree of mobility, and indications of feeding) were taken at least twice daily, once between 8.00 and 10.00 h and again between 15.00 and 17.00 h. Test prey were examined for signs of predation when the food supply was changed (every three days).

Controls. To provide experimental controls in all trials, response data were collected for larval and adult predators taken from the same rearing batch and exposed to the normal prey or confined with water only. Positive controls (PC) using the target prey were used to confirm that the predators were capable of normal feeding and development. In one of our trials, for example, *R. cardinalis* was observed to be sluggish and control beetles didn't feed on *I. purchasi.* We later discovered that those beetles were infected with a pathogen, and we had to restart the source colony. The use of such positive controls also enabled us to compare larval development rates of controls with those of individuals reared on various test species.

Because we were not able to directly measure feeding, we compared survival time when beetles were exposed to a test species to survival time with water alone. This was especially important for adults, for which – unlike larvae – there were no obvious ways to observe growth as a consequence of food intake. We reasoned that, if feeding was taking place, then beetles would live longer than starved beetles, which acted as negative controls (NC). In retrospect, it would have been useful to have also included such negative controls for larvae.

Only two treatments (T and PC) were used for testing adults against other predators because we were more interested in directly observing the interactions between the two species rather than measuring survival.

Number of insects per replicate We set the number of predator larvae per replicate at one because the immature stages of *R. cardinalis* are cannibalistic. For adults, we used a female-male adult pair in each replicate to ensure that naïve females had mated, even though males did not live as long as females. Mean survival time for adult predators was calculated for each replicate. When only one sex of the predator was available, the same sex was used for all treatments.

For species of prey, the exact number of a test species present in a trial was usually unknown because of the small size of most species, the difficulty in counting them (see Figure 5), and the fact that new prey hatched from eggs during the trials. In most cases, several individuals of different stages of each species were included in tests.

Number of trials and replicates As replication, our goal was to run 15 to 20 replicates per test species per trial and repeat a trial at least twice. Ultimately, the number of prey tested depended on their availability and that of the predator. Across all prey species the number of trials varied from one to seven ($\overline{X} = 1.88$), and the number of replicates from 3 to 31 ($\overline{X} = 12$). When the number of replicates in the trial with a given prey was low (< 7) or if the prey species occurred on many host plants, we increased the number of trials. If we knew that a given prey would be difficult to obtain a second time, we increased the number of replicates in a trial. When we only had a small number of a scarce species, trials were run even if the number of replicates was low (< 4). In all cases, we maximized the number of trials and replicates devoted to testing neonates because we considered that this was the most crucial stage to be tested.

Duration of experiments A treatment and its corresponding control(s) (together being one replicate) were run at the same time. However, because it was difficult to have enough predators ready at the same time, replicates were staggered over many days. Trials were terminated 7 days after all the individuals that had been exposed to the test prey species and the control with only water (NC) had died.

STEP 8: PREVENTING CONTAMINATION WITH SEMIOCHEMICALS

In the test arena To reduce the possibility of volatile chemicals from test insects influencing prey selection, each species and its control was placed in a different perspex cage (50 x 50 x 50 cm). Cages with *I. purchasi* were placed at the other end of the room from the treatment cages. (We were unable to keep them in separate rooms due to space constraints.) Petri dishes were recycled because of limited materials and were washed in a biodegradable and odorless detergent with a final rinse in a 1% Clorox bleach solution. The perspex cages were washed in the same manner after each experiment.

Minimizing the effects of I. purchasi *on naïve individuals* To reduce exposure of naïve neonate *R. cardinalis* larvae to chemical volatiles from *I. purchasi*, we isolated mature *R. cardinalis* adults (previously fed on *I. purchasi*) in plastic containers (11 cm dia.) with cotton balls. Isolated adults were fed honey and water, and after three days, eggs in the cotton wool were placed in a clean container for larval emergence. To obtain naïve adults, we isolated two-day old pupae, dipped them in 1% Clorox solution, and placed them in a sterile container for adult emergence. This method may not have been completely effective in eliminating volatiles from *I. purchasi*, but other solvents were not available.

Minimizing the influence of host plants on prey selection Alkaloids are sequestered by the scale *I. purchasi* from several species of Leguminosae, Aceraceae, and Menispermaceae that deter *R. cardinalis* from feeding on the scale or make it less suitable for predator development (Quezada and Debach, 1973; Mendel and Blumberg, 1991; Mendel *et al.*, 1992). Before running our trials, we checked the likely Galápagos host plants of non-target prey against a list of plant genera known to produce alkaloids. We also fed *R. cardinalis* on *I. purchasi* reared on as wide a range of host plants as possible to see if there were any plant species that influenced prey selection. To our knowledge, none of the prey species we used fed on plant species with toxic alkaloids.

STEP 9: DATA ANALYSIS—WHEN ARE TESTS RESULTS VALID?

Trials were only considered valid when more than 75% of the controls that fed on *I. purchasi* survived. We did not use any statistical method for analyzing data on larval survival because the prolonged process of feeding on prey and the existence of larval molts made it easy to detect feeding or development. Furthermore, water-only controls (NC) were not used for comparison. For adults, the average survival time was calculated for each treatment. Because the control groups fed on *I. purchasi* were terminated approximately one week after the beetles from the other treatments died, data were not normally distributed. Consequently, a Kruskal-Wallis test was used to detect significant differences in survivorship between treatment and control means. An independent sample t-test analysis was used to determine significant differences between treatments (T) with the test species and the negative controls with no food (NC) if equal variance was confirmed by the Kruskall-Wallis Test. The Mann-Whitney U test was used in the event of unequal variance. The statistics were calculated with the SPSS system (Norusis, 1993).

TEST RESULTS AND INTERPRETATION

Larvae

Results were considered valid for 16 species (from nine families) for tests with neonate larvae and for 11 species (from eight families) for tests with late instar larvae (Table 2). Test species included members in three insect orders (Homoptera, Coleoptera and Neuroptera). Larvae of *R. cardinalis* only fed on *M. similis*, a congeneric of the target pest. Only females of *M. similis* that had emerged from their protective waxy cysts were consumed. Neonate larvae lived up to 7 days ($\bar{x}$ =1.7 days, SD = ± 1.5, n = 94) on *M. similis*, but were unable to molt to second instar, suggesting that *M. similis* adults were not suitable for development. On all other prey species, *R. cardinalis* larvae died within 1 to 2 days. Because *M. similis* became unavailable in the field and could not be reared in the laboratory, only three late instar *R. cardinalis* larva were tested on this species. All three larvae completed development to the adult stage, but we were unable to observe whether they were able to develop and reproduce. Mature larvae did not feed on any other prey species offered, although they could live for up to 15 days, which was equal to the time taken for larvae feeding on *I. purchasi* to complete their development.

Although, *R. cardinalis* larvae did not feed on or kill the two predators tested (*C. cincta* and *Pentilia* sp.), on one occasion a mature larva of *R. cardinalis* and the *Pentilia* sp. were found with their jaws locked together. Conversely, larvae of the lacewing were often observed extracting the fluids from dead or dying *R. cardinalis* larvae. In addition, preliminary observations showed that *R. cardinalis* larvae did not approach a *Diomus* species (Coccinellidae) or the lepidopteran *P. rileyi.*

Adults

Representatives from two insect orders (Homoptera and Neuroptera) were successfully tested against adults of *R. cardinalis* (Table 3). Adults with prior feeding experience on *I. purchasi* were tested against eight non-target species from six families, and naïve adults were tested against six species from five families. As with the immature stages, we observed that both conditioned and naïve adult *R. cardinalis* beetles fed on females of *M. similis* that had emerged from cysts. Naïve, mated *R. cardinalis* adult pairs given emerged *M. similis* females lived significantly longer ($\bar{x}$ =10.5 days, SD = ± 3.8, n = 10, $P < 0.001$) than starved individuals (treatment NC) ($\bar{x}$ =3.8 days, SD = ± 1.0, n = 10). Moreover, 65% of the beetles survived for more than 13 days, at which stage experiments had to be terminated due to a shortage of *M. similis.* On the other hand, the longevity of beetles previously fed on *I. purchasi* and then exposed to *M. similis* was not significantly different from that of the negative control beetles (NC) fed only water. Adult beetles were unable to break open the hard waxy cysts that typically protect *M. similis* females, and the presence of the cysts in the test arena did not result in beetles living longer than individuals that were starved.

We did not observe naïve or conditioned adults feeding on other species of Coccoidea and did not find any obvious signs of feeding (such as punctured ovisacs and torn scale insects). Beetles rarely settled on test Homoptera and were very active, moving continuously in circles around the dish. Conditioned *R. cardinalis* adults tested against six additional scale insect species lived for an average of 3.1 days (SD = ± 1.3, n = 81) and did not live any longer than the controls (NC) held with water only ($\overline{X}$ = 2.7 days, SD = ± 1.0, n = 79) in 75% of the trials. Beetles tested against a new species of *Pseudoccccus* sp. #6 and *E. papillosus* lived longer than the controls within the same trial ($P < 0.05$). However, only beetles tested against *E. papillosus* lived longer ($\overline{X}$ =4.2 days, SD = ± 1.0, n = 4) than the average for conditioned beetles given only water when the trials were pooled for conditioned beetles tested against Homoptera ($\overline{X}$ =2.8 days, SD = ± 1.0, n = 99). Likewise, naïve *R. cardinalis* adults tested against three out of four species did not live any longer than controls given only water, while adults tested against the pseudococcid *P. solani* lived significantly longer ($\overline{X}$ = 6.7 days, SD = ± 0.9, n = 12, $P < 0.05$) than both their water-fed counterparts and the average for water-fed controls when data were pooled across all trials with naïve adults tested against Homoptera ($\overline{X}$ =5.4 days, SD = ± 1.8, n = 68). Adults were not observed feeding on larvae of the lepidopteran *P. rileyi* or larvae of the lacewing *C. cincta*. In contrast, adults that were weakened by a lack of food were often attacked by this neuropteran. None of the species exposed to naïve beetles were suitable for egg development, including *M. similis*. Egg laying was only observed after individuals had eaten *I. purchasi.*

Excluding the trials conducted on emerged *M. similis*, mean survival time was marginally or significantly higher for both naïve and conditioned adults fed on the test Homoptera compared to those fed only on water in 73% of the trials (n = 15). However, in all trials where Homoptera were tested, the maximum number of days an individual remained alive did not differ markedly between the controls and test species. Because we didn't find any evidence of feeding, we concluded that increased survivorship might have been because adults either fed on honeydew or attempted to feed on the test prey. It is also likely that the presence of Homoptera might have stimulated beetles to forage for longer before giving up. Significant differences in lifespan were noted between the treatments and controls in both tests with naïve and conditioned *R. cardinalis* adults. This suggests that prior feeding experience may not influence host selection. By repeating these trials we would have had a clearer idea of the response of adult *R. cardinalis* to families other than Margarodidae; however, by the time that the results were analyzed, the test species were unavailable.

PROBLEMS ENCOUNTERED WITH TESTING PROCEDURE

A summary of shortcomings and how we dealt with them is shown in Table 4. The principal setbacks encountered during the feeding trials are discussed below.

Difficulty in Locating Test Species

Our biggest problem was finding the species that we needed to test. Many of the species that were identified as potential non-target prey were found only on islands far from that where the host testing was carried out. Inter-island transport is very expensive in the Galápagos, and this

Table 4. Summary of problems and solutions encountered during feeding tests.

Shortcomings	**Our solution**	**Ideal**
Unable to determine prey range of *R. cardinalis* in the field	Literature and museum databases searched extensively. Specialists contacted.	Conduct exploratory surveys in *R. cardinalis'* native range or countries where it has been introduced.
Little known about the foraging behavior of *R. cardinalis* and factors that might influence test results.	Preliminary behavioral studies conducted. Predictions made based on current knowledge of the behaviour of Coccinellidae.	Carry out in-depth behavioral studies.
Checklist of Galápagos species incomplete.	Field surveys conducted. Deductions based on what is known from other parts of the world.	Survey extensively.
Field survey for test species limited by budget.	Ranked potential non-target species according to priority for testing. Tested species that had not been identified as potential non-targets but were from the same families as potential non-targets.	Amplify surveys.
Rearing of test species in laboratory prevented by space, budget, and quarantine constraints.	Collected material directly from field.	Rear high priority test species on plants to obtain colonies free of natural enemies and pathogens.
Difficulties locating the target prey, *I. purchasi.*	Searched far and wide on island for healthy infestations.	Maintain colonies on potted plants in cages.
Difficulties evaluating whether adult *R. cardinalis* fed on test species.	Measured survival (number of days alive) and compared this with controls fed only water.	N/A
Contaminants: insect and plant semiochemicals	Washed test arenas thoroughly and separated the arenas with the target prey from those that contained the test species. Used host plants that are not known to produet alkaloids.	Maintain test species and controls in different rooms. Wash containers with solvents suitable for eliminating volatile chemicals or use new containers. Test species on a range of host plants and without host plant.

precluded us from collecting some of the species reported from the outlying islands. Moreover, because some species had only one known collecting record (e.g. the Ortheziidae species), we could not predict the best time to collect them, so trips often failed to locate desired insects. Extended dry periods following an El Niño event caused many plants to dry out, which further exacerbated the problem, especially for testing against *R. cardinalis* adults. It also limited the range of host plants on which each non-target species could be tested and prevented us from repeating some tests. Moreover, for some species, specimen labels were very vague about the host plant (e.g., "under yellow plumed plant"!), making it difficult to locate the species.

In order to increase the number of species tested against *R. cardinalis*, we opted for a find-and-test approach, testing any likely species that we came across, even if they were introduced species. This let us increase the range of species tested against *R. cardinalis* and better determine its feeding range. Given the circumstances, we considered that even just testing species from the same family as a potential non-target species was valuable.

Keys were not available, so that once a species was located, its identification had to be confirmed by sending the specimen off to a scale insect taxonomist. Because this was time consuming and because we often needed to test the species immediately, we often tested a species before we knew what it was.

TARGET PEST AVAILABILITY

At the time our studies were initiated, *I. purchasi* was abundant in the field, and we assumed sufficient quantities could continuously be collected to feed to our *R. cardinalis* colony and run experiments. However, midway through the experiments, *I. purchasi* density declined because of drought, causing some experiments to be postponed. Additionally, some experiments were terminated early because some of the cottony cushion scales collected in the field were contaminated with mites or fungus. As a result, our colony had to be reduced in size to remove contaminants.

In retrospect, it would have been worth the investment of setting up a colony of *I. purchasi*. Although time consuming, this would have allowed us to have a continuous, uniform supply of the target pest. Maintaining the colony of *I. purchasi* under semi-quarantine conditions (i.e., in large cages) would also have eliminated contaminants.

EVALUATION OF FEEDING RANGE TESTS

DID WE TEST A WIDE ENOUGH RANGE OF POTENTIAL NON-TARGET SPECIES?

By including introduced species and a variety of native and endemic species in our tests, we were able to test neonate and mature *R. cardinalis* larvae against a wide range of species and demonstrate that *R. cardinalis* larvae have a narrow prey range.

Neonate larvae were tested against 35% (n = 17) of the homopteran species present in the Galápagos that were classified as potential non-target prey of conservation value. Mature larvae were tested against 29% of these species. Using endemic, native, and introduced species, we were able to test neonate and mature larvae against at least one species from each Homoptera family containing a species potentially at risk (Table 2). These test species included the endemic margarodid *M. similis*, which is the closest relative to *R. cardinalis*' usual prey (*I. purchasi*). Tests also included up to four species of above-ground mealybugs, the prey group most likely to be encountered by *R. cardinalis*, the group with the largest number of Galápagos endemics, and our highest priority for testing.

The smaller number of species tested with adult predators made reaching conclusions about adult prey range more difficult. Conditioned adults were tested against 29% (n = 17) of the high-risk Coccoidea, including representatives of four of the six families containing potential non-target prey. Naïve adults were tested on 23% of the high risk species and three of six families of interest. Testing of a wider range of species and repeating some trials would have been preferable, but extended dry periods following an El Niño event prevented this. We were unable to test adults on Ortheziidae, one of the closest families to the target prey. Trials with aphids were rendered invalid because of parasitization. Aphids, however, are distantly related and are unlikely to be used even as a temporary food source.

Definitive conclusions could not be reached about the extent of feeding of *M. similis*. Because only adults were tested, the possibility exists that eggs and early instars of *M. similis* might support *R. cardinalis* development; *R. cardinalis* has been shown to complete development on eggs but not adults of other genera of margarodids (Balachowsky, 1932; Mendel *et al.*, 1998). Additional studies were not considered necessary because this species is subterranean and should not be exposed to the predator.

Unfavorable collecting conditions in the field also prevented us from sufficiently evaluating the interactions of Galápagos predators with *R. cardinalis*. Because this group of non-target species was under-represented in tests, we are unable to reach any conclusions about the potential interactions between the natural enemies of scale insects and *R. cardinalis*.

THOROUGHNESS OF METHODS AND RECOMMENDATIONS FOR OTHER PRACTITIONERS

The methods employed in this study were considered to be sufficiently rigorous to answer our questions about the feeding range of *R. cardinalis*. In practice, however, the lack of baseline data on the Galápagos Homoptera made it difficult to identify all species that might be affected by the introduction of *R. cardinalis*, while a small budget limited the number of field surveys that we could carry out to collect test species. The completeness of the assessment was also limited by testing *R. cardinalis* from only one geographic area. Testing *R. cardinalis* from different geographical locations would have had the advantage of increasing the genetic variability of the test material and reducing the risks of unpredicted non-target impacts associated with introducing the beetle from a geographical area in the event that it was no longer available from the original source.

Our limited budget forced us to devise cost-effective methods for testing this predator. Initial investment in obtaining literature allowed us to understand the behavior and biology of *R. cardinalis*, which helped us to determine the most appropriate test methods to use. Testing alternative species as family-level representatives of those non-target species that could not be located allowed us to test a greater number of species and complete the trials more quickly. The rationale used here was that, as long as we could define the prey range of *R. cardinalis*, it did not matter if we could not find all the non-target species desired for testing. In retrospect, it seems clear that the order in which species in such a program are tested can also influence the number of trials that need to be carried out. By defining the feeding range of *R. cardinalis* first, one can better identify the species that might be affected (by niche overlap, intraguild predation, or competition) and thus limit the number of species that need to be tested.

Our use of field-collected specimens in this study allowed us to quickly and cheaply test a wide variety of species. Nevertheless, it would have been better to rear at least the high priority test species in the laboratory. Testing field-collected material was deemed acceptable because few Galápagos Homoptera seemed to have parasitoids or pathogens (except for aphids). Nevertheless, field parasitization of introduced species reduced the range of species tested. The use of this method in areas where parasitism is higher would not be practical. Furthermore, because we didn't rear test species on their host plants, tests could not be carried out under even semi-natural conditions (as recommended by Sands and Van Driesche, 2003). This is, however, less important for coccinellids, which appear to respond to short-range cues associated with the prey (Dixon, 2000). Furthermore, our preliminary research and the findings of other authors, showed that the size of the test arena used in our experiments was unlikely to have influenced the feeding behavior of larvae or adults of *R. cardinalis.* For new projects, we recommend that reseachers compare the behavior of the predator in different test arenas before experiments are initiated. Finally, extensive efforts should be made to minimize effects of prey or host plant volatiles or plant structural defenses.

CONCLUSIONS

We summarize our findings in terms of the questions asked by the authorities and entomologists responsible for evaluating the proposed introduction of *R. cardinalis* to the Galápagos.

- **Could *R. cardinalis* survive in the long-term on Galápagos insects?** Out of a wide range of scale insects, neonates of R. cardinalis survived only on *I. purchasi* which suggests that the predator would be unable to complete its lifecycle and survive in the long term solely on other species from the Galápagos.

- **Are Galápagos insects suitable for *R. cardinalis* reproduction?** Test results with a small range of species indicated that, in the Galápagos, *I. purchasi* is the only species that is adequate for oogenesis of *R. cardinalis.* Additional tests are necessary to confirm this.

- **Could *R. cardinalis* adults and larvae survive temporarily on Galápagos insects in times of prey scarcity?** The only test species that supported any short-term feeding was the endemic species *M. similis*, the only other Margarodidae in the Galápagos. However, field studies have since shown that the subterranean habitat of this species makes it an improbable alternate prey for *R. cardinalis* (Causton *et al.*, 2004). Test results suggest that neither young nor old larvae would be able to use above-ground Coccoidea species in the Galápagos as alternate prey. The prey range of adult *R. cardinalis* also appears to be narrow. However, additional trials are required to determine whether they are restricted to feeding on Margarodidae.

- **Does prior feeding experience influence prey selection?** Recently emerged larvae and adult *R. cardinalis* behaved the same as larvae and adults that had fed previously on *I. purchasi*, suggesting that prey selection was not influenced by prior experience with target prey.

- **Are damaging interactions likely with natives predators of scale insects?** Insufficient information due to a scarcity of necessary test insects prevents us from thoroughly evaluating the potential impact of *R. cardinalis* on native predators of Galápagos scales. However, intraguild predation and competition by *R. cardinalis* are doubtful because (1) *R. cardinalis* feeds specifically on Margarodidae and the only native predator of cottony cushion scale (the lacewing *C. cincta*) attacks larvae and weakened adults of *R. cardinalis* in captivity. Indeed, coccinellid larvae in general are susceptible to predation by lacewing larvae (Balduf, 1935; Bartlett, 1978; Sengonca and Frings, 1985; Waterhouse, 1991); (2) resident coccinellids and most other scale insect predators in the Galápagos do not feed on Margarodidae; (3) there is little habitat overlap between the prey of native coccinellids and *R. cardinalis*; and (4) *R. cardinalis* did not attack four commonly encountered species tested in these laboratory trials.

- **Is *R. cardinalis* safe to introduce into the Galápagos?** Results from our feeding range studies and risk assessment confirm the stenophagicity of *R. cardinalis* as previously reported (e.g., Quezada and Debach, 1973; Mendel and Blumberg, 1991; V. Brancatini, pers. comm., 1999).

The technical advisory committee of the CDF and the GNPS concluded that the potential detrimental effects of *R. cardinalis* on the environment and non-target organisms were minimal in relation to the immediate threat of endangered flora going extinct from damage by *I. purchasi*. Approval for *R. cardinalis*' release was granted in 2001, and over 1500 adult *R. cardinalis* have been liberated in priority areas on eight islands. Information is being gathered on the feeding behavior of the beetle in order to evaluate the effectiveness of *R. cardinalis* in reducing the target prey and its interactions with various Galápagos species.

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CHAPTER 16. EVALUATING HOST RANGE OF *LARICOBIUS NIGRINUS* FOR INTRODUCTION INTO THE EASTERN UNITED STATES FOR BIOLOGICAL CONTROL OF HEMLOCK WOOLLY ADELGID

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HEMLOCK WOOLLY ADELGID IN NORTH AMERICA

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Homoptera: Adelgidae), is a serious threat to hemlock landscape and forest stands in the eastern United States (McClure, 1996). Eastern hemlock (*Tsuga canadensis* [L.] Carr.) and Carolina hemlock (*Tsuga caroliniana* Engelm.) are very susceptible to HWA attack, and infested trees have died in as little as four years (McClure, 1991).

DISTRIBUTION OF HWA

HWA is believed to have originated in Asia (McClure, 1987), and was first observed in North America in the Pacific Northwest in the early 1920s, where Annand (1924) described it from specimens collected on western hemlock, *Tsuga heterophylla* (Raf.) Sargent, in Oregon. An earlier description in 1922 identified the species as *Chermes funitectus* Dreyfus, also from western hemlock in Vancouver, British Columbia (Annand, 1928). Annand (1928) reported that the two species were the same.

HWA is exotic to eastern North America (McClure, 1987). First collected in the eastern United States in Virginia in 1951 in an ornamental setting (Stoetzel, 2002), it has spread to forests where it currently occurs in parts of 16 states along the eastern seaboard from North Carolina to New England (USDA FS, 2004). The main front of the HWA infestation is advancing at approximately 25 km per year (McClure, 2001).

There are nine recognized species of hemlock (Farjon, 1990). Their distribution is restricted to cool, moist regions of North America and Asia in areas without either extreme winter or summer temperatures (Burns and Honkala, 1990; Farjon, 1990). In North America, hemlocks occupy two regions widely separated from each other. One is the Pacific Northwest with extensions in the northern Rocky Mountains and the Sierra Nevada Mountains of California. The other area extends from Nova Scotia west to Minnesota and south in the mountains to northern Alabama (Farjon, 1990). In Asia, hemlocks occur in Japan (though not on Hokkaido), on Taiwan, and scattered across much of the mountainous regions of eastern, central, and western China, extending westward to northwest India (Farjon, 1990).

HWA HOST RANGE

Species of hemlock in Asia and western North America are attacked by HWA but are seldom damaged. In the Pacific Northwest, HWA is not considered a forest pest, but it can weaken and kill ornamental trees (Furniss and Carolin, 1977). Tree resistance and natural enemies have been suggested as playing a role in maintaining HWA below injurious levels in these two regions (Cheah and McClure, 1996; Montgomery and Lyon, 1996). In contrast, hemlock species in eastern North American have little or no tolerance to attack by HWA. Infested trees exhibit poor crown condition, reduced terminal branch growth, and needle loss that often results in mortality when trees are predisposed to other stresses (i.e., drought) (McClure *et al.*, 2001; Souto *et al.*, 1996).

Hemlock stands in eastern North America that provide important habitats for a number of fish and wildlife species are at risk (Evans *et al.*, 1996; Quimby, 1996). Studies by McClure (1987) and Montgomery and Lyon (1996) in Connecticut, and Wallace and Hain (2000) in North Carolina and Virginia, documented a number of native or established predators associated with HWA, but they were generally found at densities too low to significantly affect populations of HWA. Because of the paucity of native natural enemies associated with HWA, classical biological control has been pursued. Since the mid-1990s, five species of Coleoptera, four in the family Coccinellidae (Tribe: Scymnini), and one in the family Derodontidae have being evaluated for potential biological control of HWA in the eastern United States (Cheah and McClure, 1998; McClure *et al.*, 2000; Montgomery *et al.*, 2000; Lu and Montgomery, 2001; Zilahi-Balogh *et al.*, 2002b; 2003ab).

BIOLOGY OF HWA

Life histories within the family Adelgidae are complicated and involve a succession of morphologically different forms and life cycles (Blackman and Eastop, 1994). Adelgids use only conifers as their host. Host alternation and cyclic parthenogenesis represent two life history characteristics within this group (Moran, 1988; 1992; Blackman and Eastop, 1994). Species may be holocyclic (host altering between primary and secondary host) or anholocyclic with no host alteration, either living on *Picea* (in which a gall may be formed) or on the secondary host (Blackman and Eastop, 1994). The genus *Picea* is the primary host in holocyclic species of both *Adelges* and *Pineus*, the two genera within the family Adelgidae. *Adelges* spp. utilize *Abies, Larix, Pseudotsuga* or *Tsuga* as their secondary host. The secondary host for *Pineus* is *Pinus* (Blackman and Eastop, 1994). HWA is known to be holocyclic in Japan, alternating between *Picea polita* and *Tsuga sieboldii* Carrière, while in North America and China it is apparently anholocyclic (Blackman and Eastop, 1994).

The life history of HWA was first studied in 1985 after its establishment in Connecticut (McClure, 1987). Subsequent studies in Virginia by Gray and Salom (1996) reported the life history to be similar, with possible differences due to a faster rate of development of the over-wintering sistens generation in Virginia. HWA completes three asexual generations on hemlock per year (McClure, 1989; 1996). Over-wintering sistens lay both alate sexuparae and apterous progrediens eggs that hatch and develop simultaneously in the spring. Sexuparae adults migrate to *Picea*, the primary host. Sexuales, the progeny of sexuparae, have not been observed to develop successfully on any species of *Picea* in North America (McClure, 1989). Progrediens remain on hemlock and lay sistens eggs that hatch and undergo an aestival diapause as a first instar nymph. Sistens resume development in the autumn and mature by February (McClure, 1989; 1996).

Life history studies in British Columbia on western hemlock revealed that the winged morph (sexuparae) is absent. This is in contrast to eastern United States (Zilahi-Balogh *et al.*, 2003a) and China (Gabriella Zilahi-Balogh, pers. observ.) and suggests a possible species complex within HWA.

OTHER ADELGIDAE IN NORTH AMERICA

There are 52 known members in the family Adelgidae in two genera, *Adelges* and *Pineus.* Five species of *Adelges* spp., including a species complex, are known to occur in North America. These are *Adelges abietis* (L.), *Adelges cooleyi* (Gillette), *Adelges laricis* Vallot complex, *Adelges piceae* (Ratzeburg), and *A. tsugae* (HWA) (Blackman and Eastop, 1994).

Adelges abietis is anholocyclic on spruce, *Picea* spp. (in North America typically *Picea abies* [L.] Karst, *Picea glauca* [Moench] Voss, and *Picea sitchensis* [Bong.] Carr.). *Picea abies* and *P. sitchensis* are not native to eastern United States. *Picea abies* is of European origin but is commonly planted as an ornamental tree in the east, while *P. sitchensis* is native to western North America. *Picea glauca* is major constituent of boreal forests in Canada. It is a minor component in northeast United States and generally confined to abandoned fields (Burns and Honkala, 1990). *Adelges abietis* is found throughout Europe, North Africa, India, and North America (Blackman and Eastop, 1994).

Adelges cooleyi typically alternates hosts between *Picea* spp. (*Picea engelmannii* Parry ex Engelm., *Picea pungens* Engelm., and *Picea sitchensis*) and Douglas-fir, *Pseudotsuga* spp. (*Pseudotsuga macrocarpa* [Vasey] Mayr and *Pseudotsuga menziesii* [Mirb.] Franco) (Blackman and Eastop, 1994). The native distribution of these conifers is limited to western North America. However, Douglas-fir has been widely planted in eastern North America as an ornamental tree (Burns and Honkala, 1990).

The *A. laricis* group occurs in Europe and North America and alternates hosts between species of *Picea* and *Larix*, or may have an incomplete cycle restricted to either the primary or the secondary host. The *A. laricis* group is made up of a complex comprising possibly up to 11 distinct species (Blackman and Eastop, 1994). Four species within this complex have been described from North America: *Adelges aenigmaticus* Annand, *Adelges diversis* Annand, *Adelges lariciatus* Patch, and *Adelges oregonensis* Annand (Annand, 1928; Blackman and Eastop, 1994). The *A. laricis* group has been recorded from *Picea abies, Picea mariana* (Mill.) B.S.P., *Picea rubens* Sargent, *Picea sitchensis* (as primary hosts), and from *Larix decidua* Miller and *Larix laricina* (*Du Roi*) K. Koch (as secondary hosts) (Burns and Honkala, 1990). *Picea mariana* occurs extensively in the boreal forests of North America. In eastern United States, it is limited to small patches in the northeast (Burns and Honkala, 1990). *Picea rubens* occurs throughout northeastern United States and in small patches in the southern Appalachian Mountains (Burns and Honkala, 1990). *Larix laricina* is a constituent of boreal forests in North America. In northeastern United States, it is commonly associated with *P. mariana* (Burns and Honkala, 1990).

Adelges piceae is anholocyclic on *Abies* spp. It is of European origin (Blackman and Eastop, 1994). In eastern North America both balsam fir, *Abies balsamea* (L.) Mill. and Fraser fir, *Abies fraseri* (Pursh) Poir are extremely sensitive to attack by *A. piceae*, often resulting in tree death (Arthur and Hain, 1984). Balsam fir has a northeastern distribution in the United States, while Fraser fir is limited to high elevations of the southern Appalachian Mountains (Burns and Honkala, 1990).

Adelges spp. known to be injurious to their hosts in eastern North America are HWA and *A. piceae*. Classical biological control programs have been targeted towards these two *Adelges* spp. in North America (Zilahi-Balogh *et al.*, 2002b, and references therein).

Of 21 described species of *Pineus* worldwide, 10 species occur in North America, and 9 of these are described from North America (Annand, 1928; Blackman and Eastop, 1994). Those known to occur in eastern United States include *Pineus boerneri* Annand, *Pineus coloradenis* (Gillette), *Pineus floccus* (Patch), *Pineus pini* (Macquart), *Pineus pinifoliae* (Fitch), *Pineus similis* (Gillette), and *Pineus strobi* Hartig. *Pineus pinifoliae, P. floccus,* and *P. pini* are injurious to either their primary or secondary hosts. *Pineus pinifoliae* alternates hosts between *Picea* spp. (*P. engelmannii, P. glauca, P. mariana, P. pungens, P. rubens,* and *P. sitchensis*) and white pines (*Pinus strobus* L. and *Pinus monticola* Dougl. ex D. Don) (Blackman and Eastop, 1994). On eastern white pine (*P. strobus*), continued heavy attack, especially on young plantations causes severe damage. Needles turn yellow, growth is reduced and occasionally trees are killed (Baker, 1972). *Pineus floccus* host alternates between *Picea* spp. (*P. rubens* and *P. mariana*) and *Pinus strobus* in eastern United States (Blackman and Eastop, 1994). Damage may be similar to that caused by *P. pinifoliae* on *Pinus*, but damage is usually not serious. On *Picea*, heavy infestations may kill the tips of branches or cause an overproduction of laterals, which leads to bushy, deformed trees (Baker, 1972). *Pineus pini* can be injurious to a wide range of *Pinus* spp. (Blackman and Eastop, 1994). In Hawaii, *P. pini* was successfully controlled with the introduction and establishment of *Leucopis (Neoleucopis) tapiae* Blanchard (Diptera: Chamaemyiidae) from Europe (Culliney *et al.*, 1988; Greathead, 1995) and *Leucopis nigraluna* McAlpine from Pakistan (Mills, 1990).

HOMOPTERA OF CONSERVATION VALUE

Paraprociphilus tessellatus (Fitch) (Aphididae) is a woolly aphid common on *Alnus* spp. and *Acer* spp. (Baker, 1972). It is of ecological importance in the eastern United States as it is one of the preferred food items for carnivorous larval stages of the butterfly, *Feniseca tarquinius* (Fabricius) (Lepidoptera: Lycaenidae). This is the only carnivorous butterfly in North America (Opler, 1998).

NATIVE OR ESTABLISHED HWA PREDATORS IN THE EASTERN UNITED STATES

Members of the family Adelgidae have few natural enemies. No parasitoids have been found in association with any adelgids. Predators have been used successfully for biological control of adelgids. Zilahi-Balogh *et al.* (2002b) reviewed the biological control efforts worldwide for the family Adelgidae. Biological control agents include Coleoptera (Coccinellidae, Derodontidae), Diptera (Cecidomyiidae, Chamaemyiidae, Syrphidae), Neuroptera (Chrysopidae, Hemerobiidae), and Homoptera (Anthocoridae). Surveys for native or established natural enemies have been conducted by McClure (1987) and Montgomery and Lyon (1996) in Connecticut and by Wallace and Hain (2000) in North Carolina and Virginia. Members of the families Cecidomyiidae, Syrphidae, and Chrysopidae were found associated with HWA by McClure (1987), but densities were too low to have any significance in reducing adelgid populations. Surveys by Montgomery and Lyon (1996) on HWA infested eastern hemlock growing in stands with eastern white pine and Scotch pine (*Pinus sylvestris* L.) recovered *Scymnus suturalis* Thunberg (Coleoptera: Coccinellidae), *Laricobius rubidus* LeConte, and a brown lacewing (Hemerobiidae). On *P. strobi*-infested eastern white pine, *S. suturalis,* and *L. rubidus*, *Leucopis* (*Neoleucopis*) *obscura* Haliday (Chamaemyiidae), and a *Tetraphleps* spp. (Anthocoridae) were recovered. Both *S. suturalis* and *L. rubidus* were abundant on both pine and hemlock (Montgomery and Lyon, 1996). *Scymnus suturalis* is of European origin and introduced into Michigan in the 1960s (Montgomery and Lyon, 1996). Surveys by Wallace and Hain (2000) in three forest sites over two years in northern North Carolina and southern Virginia on HWA-infested eastern hemlock collected *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae); *Chrysoperla harrisii* (Fitch) (Neuroptera: Chrysopidae); *Hemerobius humulinus* L. and *Hemerobius* sp. (Neuroptera: Hemerobiidae); *Aphidoletes abietis* Kieffer, *Aphidoletes aphidimyza* Rondani, *Aphidoletes* sp., *Lestodiplosis* sp., and *Trisopsis* sp. (Diptera: Cecidomyiidae); *Leucopis* sp. (Diptera: Chamaemyiidae); unspecified Syrphidae; and *L. rubidus* (Coleoptera: Derodontidae). Predators were collected in very few numbers over both years. Overall, *H. axyridis,* Neuroptera, and Cecidomyiidae were the most abundant. In cage exclusion experiments, there were no significant predator effects (Wallace and Hain, 2000).

Laricobius rubidus is the only native *Laricobius* spp. in eastern North America and therefore of ecological value. Because there is some overlap in the diets of *L. rubidus,* we expect some competition between these two congeners and the introduced *Laricobius nigrinus* Fender in the eastern United States. However, the inability of *L. nigrinus* to complete development on *P. strobi* suggests that the two congeners will not compete on the primary host of *L. rubidus* (Zilahi-Balogh *et al.*, in press).

HWA PREDATORS: THE GENUS LARICOBIUS

Laricobius is one of four genera in the family Derodontidae that inhabits relatively humid forests in temperate regions (Lawrence and Hlavac, 1979). Members in the genus *Laricobius* are predacious on woolly adelgids (Homoptera: Adelgidae). In contrast, the remaining three genera feed on fungi or on the by-products of fungal metabolism (Lawrence and Hlavac, 1979; Bright, 1991). The genus *Laricobius* is poorly studied. Small size (< 3 mm), cryptic habits, and activity concentrated in the cooler months of the year make member of this genus rare in collections.

Eleven species of *Laricobius* are described from the Holarctic. Since Lawrence and Hlavac (1979) reviewed the Derodontidae, two new species have been described from Siberia (Nikitsky and Lafer, 1992), as well as two species from Nepal (Háva and Jelínek, 2000; Jelínek and Háva, 2001), and one species from China (Háva and Jelínek, 1999).

LARICOBIUS IN NORTH AMERICA

In North America, there are four described species of *Laricobius*. They are *Laricobius erichsonii* Rosenhauer, *Laricobius laticollis* Fall, *L. nigrinus*, and *L. rubidus*. *Laricobius erichsonii* is native to Europe and was introduced into North America in the 1950s and 1960s as a biological control agent of *A. piceae*, while the three remaining species are native to North America (Hatch, 1962; Lawrence, 1989; Bright, 1991).

The preferred host of *L. erichsonii* is *A. piceae*, but other hosts include *A. cooleyi*, *Adelges nusslini* Borner, *Pineus pineoides* (Cholodkovsky), and *P. strobi* (Lawrence and Hlavac, 1979). *Laricobius laticollis* has been collected from Douglas-fir, *P. menziesii*, in the Pacific Northwest (Brown, 1944; Lawrence and Hlavac, 1979), but no prey associations have been reported to date. *Laricobius rubidus* is native to eastern North America with a distribution extending from the District of Columbia north to New Brunswick, west to Michigan (Brown, 1944; Lawrence, 1989), and more recently, as far south as North Carolina (Wallace and Hain, 2000). The primary host of *L. rubidus* is the pine bark adelgid, *P. strobi*, but *A. piceae* (Clark and Brown, 1960) has also been reported as a host. *Laricobius rubidus* has also been infrequently collected from HWA-infested eastern hemlock in Connecticut (Montgomery and Lyon, 1996), northern North Carolina, and southern Virginia (Wallace and Hain, 2000). Studies by Zilahi-Balogh *et al.* (in press) demonstrated that HWA is a suitable host for *L. rubidus*. Larvae fed a diet of only HWA completed development to the adult stage. In addition, there were no significant differences in development time of *L. rubidus* fed a diet of HWA or *P. strobi*. However, in a paired-choice test, oviposition by *L. rubidus* was more than six times greater on *P. strobi* than on HWA, indicating an ovipositional preference for *P. strobi*.

LARICOBIUS NIGRINUS

Laricobius nigrinus (Fender, 1945) has a known distribution in British Columbia, western Washington, Oregon, and northern Idaho (Hatch, 1962; Lawrence, 1989). The distribution of this predator appears to coincide with the distribution of western hemlock (Burns and Honkala, 1990). *Laricobius nigrinus* was first observed in close association with HWA populations on western hemlock in British Columbia in the early 1990s (Leland Humble, Canadian Forest

Service, Victoria, Canada, pers. comm.). We selected this beetle for evaluation as a candidate biological control agent for HWA for two reasons: (1) *L. nigrinus* has been found consistently in association with HWA in the Pacific Northwest; and (2) HWA is not considered a forestry pest in the Pacific Northwest. We hypothesized that *L. nigrinus* may play a role in regulating HWA abundance in the Pacific Northwest and therefore warranted investigation as a candidate biological control agent of HWA in the eastern United States.

The life history of *L. nigrinus* was studied over two years in British Columbia (Zilahi-Balogh *et al.*, 2003a). *Laricobius nigrinus* is univoltine. Females lay eggs singly within the woolly ovisacs of HWA from January to May. Onset of egg laying by *L. nigrinus* coincides with egg laying by the over-wintering (sistens) generation of HWA. After hatching, larvae feed on the eggs of HWA. On completion of feeding, mature larvae migrate to the soil to pupate. Emergent adults remain in the soil in aestival diapause, resuming activity in the autumn at about the same time that aestivating nymphs of HWA (sistens) resume development (Zilahi-Balogh *et al.*, 2003a). In summary, *L. nigrinus* attacks two generations of HWA. Adults feed on the developing sistens from the time they become active in the autumn, while larvae feed on the eggs laid by over-wintered sistens. The phenology of *L. nigrinus* in Virginia is similar to that in British Columbia (Lamb, pers. comm.).

RESEARCH RATIONALE

Host range testing was conducted by Zilahi-Balogh *et al.* (2002a) under quarantine in Virginia to determine if *L. nigrinus* behaved similarly to other congeners and had a preference for HWA over other adelgids. Six species of prey (Homoptera) in three families (Adelgidae, Aphididae, and Diaspididae) were evaluated in host specificity tests. Within the family Adelgidae, we selected *A. piceae*, *A. abietis*, and *P. strobi*. We would have liked to include *A. cooleyi* and *A. lariciatus* in our host range tests, but were unable to collect *A. cooleyi* in numbers high enough for evaluation. We attempted to use *A. lariciatus*, which attaches to the needles of its host plant (*L. decidua*), but found that *L. decidua* needles desiccated and dropped from the twigs in a very short period of time (1-2 d) and therefore were not suitable in a 3-day bioassay. We broadened our taxonomic scope and selected other Homoptera in two families: Aphididae (*Cinara pilicornis* [Hartig], *Myzus persicae* [Sulzer]) and Diaspididae (*Chionaspis pinifoliae* [Fitch]). We used these taxa over other Aphidoidea because of their availability. *Myzus persicae* does not feed on Pinaceae and therefore would never be encountered by *L. nigrinus* in the field; however, it was found infesting potted sweet pepper plants (*Capsicum frutescens* L. var. *grossum* cv. California Wonder) in the greenhouse and therefore was available for use. We would have preferred to evaluate a scale insect that feeds on *Tsuga* spp.—e.g., *Fiorinia externa* Ferris or *Nuculaspis tsugae* (Marlatt) (Diaspididae)—over *C. pinifoliae* but were unable to collect either species from hemlock in high enough numbers to evaluate. In retrospect, the woolly alder aphid, *P. tessellatus,* should have been considered in host range testing as it is a preferred host for the larvae of the carnivorous butterfly *F. tarquinius.* However, its ecological importance was inadvertently overlooked at the time. Current host range testing is including this species.

Before host range testing could be initiated, potted tree saplings infested with their associated insects were grown in an outdoor tree nursery. With the exception of *A. piceae*, test prey were collected from ornamental trees near Blacksburg, Virginia, or from the Blacksburg Ranger Forest District in Giles and Montgomery counties, in Virginia (Zilahi-Balogh *et al.*, 2002a). *Adelges piceae* was obtained from Fraser fir trees dug up and potted from a Christmas tree plantation in Avery County, North Carolina. *Chionaspis pinifoliae*, HWA, and *A. abietis* were field collected from their associated tree at the appropriate stage before tests and held at 4°C in moistened floral foam (Oasis®) before use.

The egg stage was used in all tests for members in the family Adelgidae and Diaspididae. Eggs of adelgids are typically laid in a mass by a sessile female and surrounded by flocculent material (waxy/woolly filaments). This stage was selected because we found *L. nigrinus* females laying eggs in the woolly ovisacs of HWA. HWA differs from the other three adelgids tested in that it breaks aestival diapause in late September/October, develops throughout the winter, and begins to lay eggs in February (McClure, 1987). In contrast, the other adelgids used in our host range tests over-winter as early instar nymphs and begin to lay eggs in the spring when buds begin to break on their host tree (April or May) (Gambrell, 1931; Friend and Wilford, 1933; Baker, 1972; Arthur and Hain, 1984; Johnson and Lyon, 1991; USDA, 1985). The challenge was synchronizing development of the various adelgid species with that of HWA. This was achieved by moving adelgid-infested potted trees from the outdoor nursery into a greenhouse (at approximately 24°C) beginning in January to accelerate development before being used in tests. Prey species in the family Aphididae were tested at the early instar nymphal stage. Prey species in the family Adelgidae and Diaspididae remain attached to their host plant once crawlers settle; excess individuals were removed from the host plant with fine forceps when numbers exceeded those required for a particular test. Individuals of the two aphid species used were transferred onto or removed from their respective host plant with a fine brush to attain the appropriate number on the host plant cutting.

HOST SPECIFICITY TESTS

Host specificity tests conducted by Zilahi-Balogh *et al.* (2002a) were of two types – host acceptance and host suitability. Host acceptance tests determine whether a candidate biological control agent will feed and/or oviposit on a host. Host suitability tests determine whether the agent is able to complete development to the adult stage and produce viable offspring on a particular host (Kok *et al.*, 1992). Host suitability tests therefore are more crucial in determining potential host range.

Adult predators (*L. nigrinus*) used in these tests were field-collected from western hemlock in British Columbia in the early spring to ensure that females were gravid and then shipped to a USDA approved quarantine facility at Virginia Polytechnic Institute and State University, Blacksburg, Virginia. Insects were maintained on field-collected, HWA-infested, eastern hemlock twig cuttings. Tests utilizing immature stages were the progeny of field-collected females.

HOST ACCEPTANCE

Oviposition tests. Both no-choice (single-prey) and paired-choice oviposition tests were conducted to evaluate the effect of prey type on acceptance and preference by *L. nigrinus* females for oviposition. All tests were conducted in 14 x 2.5 cm plastic petri dishes. One male-female pair was placed in a petri dish with either one bouquet of associated host plant twigs housing the test prey (no-choice test) or two adjacent bouquets of host plant with associated prey (paired-choice test). A bouquet was made up of two to four terminal tip branches (10-12 cm length) of prey-infested host plant held together by wrapping the cut end with parafilm to prevent the twigs from drying out. In the paired-choice tests, HWA was paired with each of the six test prey. The same numbers of prey (50 to 60 individuals per bouquet) were used in each test. Duration of each test was three days. The number of *L. nigrinus* eggs deposited on each plant bouquet was counted at the end of each test (Zilahi-Balogh *et al.* 2002a). A 3-day test was selected based on preliminary trials that showed that three days was a long enough interval to get a treatment effect without resulting in host plant desiccation or having to add additional prey.

Adult feeding test. Prey acceptance by adult *L. nigrinus* was examined in a single-prey feeding experiment using eggs of the four adelgid species, HWA, *A. abietis*, *A. piceae*, and *P. strobi*. Even though *L. nigrinus* adults preferentially feed on nymphs and adult stages of adelgids, eggs were selected to test because they are uniform in size within a species and similar in size amongst species. Adult *L. nigrinus* were starved for 12 hours and then placed individually in 50 x 9 mm petri dishes containing one of four prey types attached to sections (< 5 cm) of host plant. Numbers of a test species were counted before introduction of the predator. After 3 days, adult beetles were removed and the number of eggs that remained were counted (Zilahi-Balogh *et al.* 2002a).

HOST SUITABILITY

Development and survivorship of *L. nigrinus* were followed from the egg to adult stage on all test prey except *M. persicae*. We did not evaluate *M. persicae* because it was the only test prey on which *L. nigrinus* females did not oviposit. *Laricobius nigrinus* eggs (ca 24 h old) were transferred individually onto test prey in petri dishes as described above in the adult single-prey feeding test. The stage of test prey used was similar to that described for the oviposition tests. Egg hatch was followed daily. Other stages were examined daily or every other day for survivorship until adult emergence. Fresh prey was added each time an individual larva was examined. Larval molt was determined by recording the presence of an exuvium. Once the pre-pupal stage was reached, moistened sterilized peat was placed at the base of each petri dish and acted as a pupation medium. The pre-pupal stage was determined to be the stage that mature larvae left the twig with abundant prey and appeared to be actively searching for a suitable pupation site (Zilahi-Balogh *et al.* 2002a).

RESULTS AND DISCUSSION

Host Acceptance

Oviposition tests In both the no-choice and paired-choice oviposition tests, *L. nigrinus* females laid significantly more eggs in HWA ovisacs than on plants bearing other adelgid and non-adelgid species of Homoptera (Zilahi-Balogh *et al.* 2002a). No eggs were laid on sweet pepper with *M. persicae* and very few eggs were laid on host plants with the other non-adelgid homopterans in the no-choice tests (Zilahi-Balogh *et al.* 2002a). In the paired-choice tests, no eggs were laid on host plant twigs with the non-adelgid test prey *C. pinifoliae* (Diaspididae), *C. pilicornis* (Aphididae), and *M. persicae* (Aphididae). Oviposition was more than five times greater on HWA than on *A. piceae*, *A. abietis*, or *P. strobi* in the paired-choice tests. These differences indicate an ovipositional preference for HWA over these other adelgids (Zilahi-Balogh *et al.* 2002a).

Adult feeding test In this no-choice feeding test, eggs of all the test adelgids were fed on by adult *L. nigrinus*. Significantly more eggs of HWA were consumed than eggs of the *A. piceae* and *P. strobi*, but not of *A. abietis*. Though not statistically significant, *L. nigrinus* adults consumed on average twice as many HWA eggs (48.4) than *A. abietis* eggs (24.7) (Zilahi-Balogh *et al.* 2002a).

Host Suitability

Laricobius nigrinus only completed development to the adult stage on a diet of HWA. *Adelges piceae* and *P. strobi* supported larval development to the fourth instar, providing evidence of larval feeding, but did not support further development. Larvae provided with only *A. abietis*, *C. pilicornis*, or *C. pinifoliae* did not survive beyond the first instar.

A summary of test results on oviposition, feeding and larval development indicate that *L. nigrinus* has a narrow host range (Table 1). Although adult feeding tests showed some feeding on other adelgid species in addition to HWA, larval development tests showed that *L. nigrinus* only completed development to the adult stage on HWA. Therefore, these other adelgid species are not suitable hosts. Maintaining *L. nigrinus* on HWA prior to and between tests may have introduced bias toward HWA in the oviposition and feeding tests, but development to the adult stage only occurred on HWA. No artificial diet is available for *L. nigrinus*.

SUMMARY

The two types of laboratory studies conducted (host acceptance and host suitability tests) reveal that *L. nigrinus* has a narrow host range. It feeds and oviposits on members in the family Adelgidae but prefers HWA over other adelgids. In addition, this predator only completed

Table 1. Summary of Results of Acceptance and Suitability Tests of Homoptera Prey Screened as Hosts of *Laricobius nigrinus*

	Acceptance[a]		**Suitability**	
Test Species	**Oviposition**	**Adult Feeding**	**Larval Development**	**Final Host Status**[b]
Adelgidae				
Adelges tsugae	+	+	+	Yes
Adelges piceae	+	+	-	No
Adelges abietis	+	+	-	No
Pineus strobi	+	+	-	No
Aphididae				
Cinara pilicornis	+	x	-	No
Myzus persicae	-	x	x	No
Diaspididae				
Chionaspis pinifoliae	+	x	-	No

[a] + = positive response on test prey; - = negative response on test prey; x = test not conducted;
[b] Whether the species could serve as a host to *L. nigrinus*.
Taken from Zilahi-Balogh *et al.*, 2002a.

development to the adult stage on HWA (Zilahi-Balogh *et al.* 2002a). Results from our host range tests (Zilahi-Balogh *et al.* 2002a) are supported by field observations made in the native range of *L. nigrinus* (Zilahi-Balogh *et al.* 2003a) and taxonomic and ecological information on the genus *Laricobius* (Franz, 1958; Clark and Brown, 1960; Lawrence and Hlavac, 1979; Lawrence, 1989).

Laboratory studies were followed up with caged field studies in a natural forest setting in Virginia with *L. nigrinus* on HWA infested eastern hemlock (Lamb *et al.*, 2002). These studies demonstrated that: 1) HWA populations on hemlock branches exposed to *L. nigrinus* suffered significantly higher mortality than branches without predators, and 2) *L. nigrinus* introduced into cages in the autumn survived (76% survival rate) and reproduced the following spring (Lamb, pers. comm.).

In conclusion *L. nigrinus* is host specific to the family Adelgidae and prefers HWA over the other adelgids tested. The possibility that a few non-target species of Adelgidae may be attacked must be balanced with the potential benefit that comes with control of HWA. In September 2000, the Animal and Plant Health Inspection Service, United States Department of Agriculture, approved the field release of *L. nigrinus* in the eastern United States.

RECOMMENDATIONS

One of the most important aspects in laboratory host range testing is learning how to rear healthy colonies of both predator (natural enemy) and prey. If not much is known about the natural enemy, the researcher needs to spend the time observing how the species responds to various environmental conditions. One of the biggest challenges in rearing *L. nigrinus* was determining what environmental conditions to use to maintain adults that undergo an aestival diapause. Spring-like conditions (15°C, 12:12 [L:D]) were optimal for ovipositing adults and for larval development (Zilahi-Balogh *et al.*, 2003a), but were unsuitable for aestivating adults because they resumed activity approximately two months earlier than those in the wild. We experienced high mortality with early emerging *L. nigrinus* fed aestivating HWA as well as alternative food sources including *A. abietis* nymphs removed from galls, honey, and Wheast®—a ladybug and lacewing diet (from Planet Natural, Bozeman, Montana). It is important to study all aspects of the biology and behavior of a biological control agent. Not only is this information useful in comparing attributes between wild and laboratory-reared populations, but is useful for developing mass rearing protocols.

Choice of testing arena and environmental conditions used need to be considered in the design of host range tests (Sands and Van Driesche, 2003) as the natural enemy may behave differently under different conditions. Bioassays that resemble conditions that the natural enemy would encounter in nature are the simplest to interpret. In retrospect, our petri dish bioassays might not have been the most appropriate arena to use for a predator that moves in three-dimensional space, such as a forest environment. Utilizing a cage as an arena that allows the predator to search vertically as well as horizontally might have been more appropriate, despite the small size of the predator.

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CHAPTER 17. CONCLUSIONS

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While many areas of design for host range testing of carnivorous arthropods are still in flux, the following four points emerge in the mind of the senior editor as ones of particular importance for consideration in planning testing programs (points 1 and 2) or where important undecided theoretical issues exist (points 3 and 4).

Point 1: The species-rich, poorly known insect faunas of the world often make constructing representative test lists of native taxa extremely difficult.

Problem There are approximately 10 to 30-fold more species of insects than plants. While most non-tropical floras have nearly all of their species described, the same is not true of insects. Similarly, molecular phylogenies of plant groups are more common and more complete than for insects. These facts mean that if carnivorous insects are being introduced to continents, particularly ones with subtropical or tropical zones, there will be huge gaps in knowledge regarding native insects related to the target pest are that occur in the region. The smaller faunas of islands makes consideration of the impacts of biological control introductions on native insects of islands more tractable (see Causton, this volume, for an example in which impact of an introduction to the Galápagos was assessed relative to the islands' native insects). Comprehensive screening of the native species of a region is thus rarely possibly, even at the subfamily or tribe level. Rather, the fundamental host range needs to be determined by testing native species from the same genus as the target pest (if any exist), together with various species in native genera of the same tribe or subfamily as the pest. The lack of modern phylogenies for many insect groups, however, will make this task harder for insects than for plants as it may not be clear which genera (of perhaps dozens or more) are most closely related to genus of the pest.

A further complication is that, even for those species that have been described taxonomically, there may be little or no literature about their exact distributions, habitats, host plants, biology, or habits. This may make it impossible to find them for use in host range tests (see Combs, this volume for an illustration of this point), or too difficult to rear them.

Potential solution Government support for taxonomic revisions and natural heritage studies of families with large numbers of pest species worldwide (and hence the groups most likely to furnish new invasive pests) are the means by to reduce this problem. Taxonomists, working with economic entomologists, could identify high priority taxonomic groups (and countries), which could then be studied in anticipation of future need.

Point 2: The inability to store test species with minimal maintenance (in contrast to the ease of storage provided by potted plants or seeds) forces testing programs for carnivorous arthropods to use field collected individuals (of the herbivorous test species) and to limit the test list severely to a few highly representative species.

(1) Use of field-collected individuals in laboratory tests.

Problem Field-collected individuals are used in laboratory tests because they are available and did not have to be reared (or were species that could not be reared). Basing a testing program around this approach (see Causton and also Fuester *et al.* in this volume) results in two problems. First, the list of species actually tested is at risk of being unbalanced as inclusion is based on opportunistic availability more than planning. Second, some test results may, later, have to be discarded (see Causton) because the test individuals turned out to have been previously parasitized or diseased.

Solution A better selection of test species may be possible if internet resources are used to link the researcher to large numbers of other entomologists who may have access to additional, desired species. The researcher could, for example, post lists of needed species, with alternative suggested species for each, to email lists or websites.

To reduce levels of contamination in field-collected species, organdy sleeves might be placed over colonies of desired species (at least for groups like aphids, scales, whiteflies, etc) to promote the development of colonies with lower rates of parasitism. Such partially protected field-reared insects could later be harvested as needed for tests. This approach would be less practical if such insects were only found in remote or difficult to rear locations.

(2) Picking highly representative species.

Problem Randomly selected members of a genus or tribe often must be used to represent their entire group. The validity of the assumption that "host suitability" is some quality that is broadly shared and gradually is diluted and lost with decreasing closeness to the target pest needs to be tested. An alternative model might be that suitability (for parasitoid oviposition, especially) changes abruptly among even closely related species.

Solution To determine if the assumptions on which this approach is based are warranted or not, a boot strap testing approach ought to be applied to several systems as test cases. For a particular parasitoid, the native species nearest to the normal host could be identified and then four or five randomly selected groups (each a randomly selected list of test species) subjected to host range testing. If randomly selected species are representative of their taxonomic higher groupings, each group of randomly chosen species should yield similar estimates of the parasitoid's host range. If suitability varies less gradually, then more variation in predictions is expected from data among test groups.

Point 3: Unlike herbivores, which at least at times encounter several host plants in the same local area (in some cases side by side), parasitoids and predators are more likely to encounter potential hosts or prey one species at a time and thus live in a no-choice world.

Problem For herbivorous weed biocontrol agents, prior experience with the target weed and the opportunity to chose between the weed and a nontarget plant in the field is generally assumed. Because plants may at times (but clearly not always) grow in stands of mixed host species, this model does represent part of the world in which weed biocontrol agents search for hosts. Because of this, weed biocontrol scientists have shown a strong preference to use results from choice tests to estimate likely host ranges of herbivores being considered for introduction. However, even herbivores may not have such choices at all times: they might, for example, disperse into geographic regions where nontarget relatives of the target weed, but not the weed itself, are found.

For carnivorous arthropods searching for hosts or prey, resources are even more likely to be encountered one species at a time. Also, for parasitoids, it has been extensively shown that previous contact with the usual host decreases acceptance of other potential hosts (see Withers and Barton Browne, this volume). Both of these facts argue against using choice tests to predict risk to native test species from carnivorous arthropods proposed for introduction. Rather, it seems better to rely on no-choice tests.

Solution The best estimates of parasitoid host range seem likely to result from the testing of naïve, gravid females in a large test arena with moving air, in which each test species is presented separately and on the test herbivore's typical host plant. Negative results in such tests are validated by positive response of the same female parasitoid in an immediately following test in the same arena with the target pest on its typical host plant.

Point 4: The relative value of host taxonomy vs. the herbivore's host plant as a predictor of host range is likely to vary between groups of carnivorous arthropods.

Problem For parasitoids, successful host use requires both host location and host suitability. Factors determining the detectability of a host by a parasitoid include the presence of volatile compounds, sometimes from the host alone (e.g., its pheromones), but often from the herbivore's host plant (volatiles emitted when the plant is fed on by the herbivore). Suitability of a host for a parasitoid (at least for koinobiont species) turns on the ability of the parasitoid's venoms, teratocytes, and symbionts to suppress the host's immune system. For idiobiont parasitoids (external parasitoids or parasitoids of stages like eggs that lack immune systems), suitability does not require suppression of an immune system, but rather will depend on the nutritional adequacy of the host's tissues.

These differences in biology are likely to strongly influence the relative value of two important potential predictors of a parasitoid's host range: (1) taxonomic relatedness of a test species to the normal host and (2) the similarity of the volatile blends emitted by the test species when feeding on its normal plant host to the volatile blend from the normal host when it feeds on its normal plant.

Solution For koinobiont parasitoids, how close a test species is taxonomically to the target pest is likely to be the best predictor of risk. So, test species should be selected by taking native species first from the same genus, then tribe, subfamily, etc., until the limits of the host range are discovered.

For idiobionts and predators, attraction to plants or restrictions to particular habitats might be a stronger factor shaping host ranges than is host taxonomy. Some parasitoids of leafminers, for example, attack hosts in several insect orders provided the leafminers are on the right sort of host tree (such as cherry) and have the right general mine shape and position (such as a blotch mine on the underside of the leaf). For such species, insects closely related to the target pest may not be hosts at all if they occur on differ types of plants or make differently shaped or positioned mines.

Continued debate on these and other points are needed to derive an effective system for predicting host ranges of carnivorous arthropods. The methods used for herbivorous arthropods, while instructive as a place to begin, do not provide an effective template. At the 2nd ISBCA meeting in Davos, Switzerland in September, 2005, discussion of these issues will continue.

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